GROWTH, DEVELOPMENT, AND REPRODUCTIVE INVESTMENTS IN HONEY BEE COLONIES

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Michael Lee Smith

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GROWTH, DEVELOPMENT, AND REPRODUCTIVE INVESTMENTS

IN HONEY BEE COLONIES

Michael Lee Smith, Ph. D.

Cornell University 2017

Chapter 1 (published in *Insectes Sociaux*) describes the pattern of honey bee colony growth and development, from colony founding through colony death. This broad-scale sociometric study tracked multiple colony parameters, including worker and drone populations, comb area and use, swarming rate, and colony death. This chapter forms the natural history foundation upon which future investigations were built.

Chapter 2 (published in *Naturwissenschaften*) is about the colony parameters that trigger workers to begin building drone comb, the first reproductive investment. By experimentally increasing individual colony parameters, we show that workers only begin to invest in reproduction once the colony surpasses a threshold colony size. This led us to investigate how workers detect that their colony is above this reproductive threshold.

Chapter 3 (published in the *Journal of Experimental Biology*) combined the observational with the experimental. The goal of the observational study was to monitor and compare cues in small and large colonies to see which cues reliably change with colony size, and how. In the experimental study, we manipulated three

cues that workers might use to sense that their colony has enough workers to invest in drone comb: (1) worker density, (2) volatile pheromone level and (3) nest temperature. We found that contact rate increases with colony size, and that worker density is less variable in large colonies. When colonies were experimentally manipulated to increase worker density, worker bees responded by producing more drone comb. This demonstrates that workers use worker density to assess colony size, although the mechanism that they use to assess worker density remains uncertain.

Chapter 4 (published in *Behavioral Ecology and Sociobiology*) used accelerometers to measure comb vibrations, while varying the number of bees on the comb. While comb vibrations did decrease with increased worker number, the damping was not sufficiently large to cue workers of their colony's size. We did, however, show that it is not the bees' mass that damps vibrations, but is likely their behavior. This damping may reduce background noise, and improve the comb landscape for substrate-borne communication.

BIOGRAPHICAL SKETCH

Michael L Smith was born in 1987, with a bit more energy than a child should have. In 1995 his family returned to Panama, where Michael spent his childhood exploring the jungle, tide pools, and planting over 1000 trees as a hobby. Africanized bees kept him from beekeeping in Panama, but he did keep a colony of *Tetragonisca angustula* in a log behind the house. In 2004, Michael attended the United World College of the Atlantic, in Wales, where he would complete his last two years of high school thanks to a Davis Scholarship. While in Wales, he had a serendipitous encounter with a local beekeeper, Chris Adam, who introduced Michael to beekeeping. He was immediately enthralled, and began a beekeeping club at Atlantic College, which continues to this day. Michael went on to Princeton for his undergraduate degree in Molecular Biology, completing his thesis in the lab of Mark Rose and Alison Gammie. He incorporated honey bees into his degree whenever possible, and founded the Princeton Bee Team (still active), but it was at Wellesley College where Michael first immersed himself into honey bee research. His sister, Annie, was working in Heather Mattila's Lab at Wellesley, when two assistants quit mid-summer. Heather asked if Michael would be willing to come work in the lab, and Michael enthusiastically hopped onto the next plane from Panama to Boston. In Heather's lab, Michael became convinced that he would combine his interest in biology with his passion for honey bees. After graduating from Princeton in 2010, and spending another summer working for Heather Mattila, Michael went to Wageningen University, in the Netherlands, to work on disease transmission in honey bees. In 2011, Michael began his PhD in the Department of Neurobiology and

Behavior at Cornell University, in Tom Seeley's lab. An initial flood of bee puns may have startled Tom, but it set the stage for an excellent working relationship and friendship (*RIGHT* TOM?!?). After trying out a few different projects, Michael devoted his time to understanding developmental transitions in honey bee colonies, especially the building of drone comb, the first reproductive investment. While focused on his thesis, Michael did not shut himself off from the occasional side project, including those that connected him back to his original beekeeping roots. He began a beekeeping club at Cornell, which is still active, and maintains the College Beekeeper website to help students at other institutions start beekeeping clubs of their own. He also met a postdoc in the neighboring department whose apartment was relatively clean, and thus suitable as a mate. In 2017, the apple trees that Michael planted upon arrival to the Liddell Field Station began to bear fruit, which meant that it's time for Michael to graduate. Thanks to the Simons Foundation, through the Life Sciences Research Foundation, Michael will be conducting postdoctoral research in Iain Couzin's Group at the Max Planck Institute for Ornithology / University of Konstanz, where he promises to do his best to beehave.

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A number of people are probably unaware that I sneakily extracted advice, guidance, and inspiration during our encounters. If you thought it was all fun and games, you were duped. General “buena gente” faculty include: Robin Davisson and David Skorton, Andy Bass and Midge Marchaterre, Jesse and Elizabeth Goldberg, Willy Bemis and Betty McGuire, Robin Seeley, Chris Schaffer, Kraig Adler, and Walt Koenig. Even when socializing, I felt that I was benefitting from our interactions.

Heather Mattila gave me the opportunity to immerse myself into honey bee research, and for that I am eternally grateful. Marla Spivak, Gard Otis, Madeleine Beekman, and Ben Oldroyd have all been excellent sources of honey bee wisdom. My work with the GC-FID and GC-MS are not part of the thesis, but that’s certainly not for a lack of trying, or expert assistance. Andre Kessler, Rob Raguso, Katalin Boroczky, Callum Kingwell, Stephen Martin, and Brian Mlodzinski all helped with training, sample processing, and managing explosions along the way. Mike Sheehan joined NBB later on, but he helped me understand how important it is to frame your

research. Joe Fetcho was a frequent subject of my pranks and jokes. Even so, he always gave honest and useful perspective from the first floor. I still have yet to receive one of his darling Valentine's Day cards.

I was fortunate to have mentors in neighboring departments, and at other institutions. Mark Rose and Alison Gammie, Brian Danforth, Scott McArt, Anurag Agrawal, Bruce Land, Simon Garnier, Barrett Klein, Stephen Pratt, and Bob Jeanne all helped at different times and in different ways. Walter Tschinkel set the standard for research grit, and he knows how to mix a proper cocktail. Kelly Zamudio and Monica Geber taught the DDIG course. Next to statistics, it is the most useful course I've taken. Bill Wcislo was always willing to host me at STRI for my yearly writing retreat, despite my semi-illegal status. I benefitted greatly from my time at STRI, including frequent discussions and outings with David Roubik, Harilaos Lessios, Hermogenes Fernandez, Paola Galgani, and John Christy. Egbert Leigh was the inspiration for the Memorial Grill Pit at Liddell. Who says you cannot be remembered while you are still alive?

I have been fortunate to receive funding throughout my PhD, especially from the National Science Foundation's Graduate Research Fellowship Program and the Doctoral Dissertation Improvement Grant. Other sources include the Centennial Pollinator Fellowship from the Garden Club of America, the Andrew Mellon Grant, Neurobiology and Behavior's research support, the Dyce Recycling Researcher Award, and Tom Seeley's kindness. Kirstin Petersen was extremely generous to fund my final semester. Writing a grant with Kirstin was a perfect insight into how PI's make research a reality.

It was Chris Adam that was kind enough to introduce bees to a high school kid that showed an interest. Beekeepers in general have been a source of inspiration, encouragement, and comic relief. I thank Bonnie and Gary Morse, the Eastern Apicultural Society, the Finger Lakes Beekeepers Club, the Southern Adirondack Beekeepers Association, and Bee Audacious, for allowing me to share my research, even early on.

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If I had grown up with Ian Kyle, we might have both spent more time in jail. He has a special ability to make anything happen, and we both share an enthusiasm for the exorbitant, best exemplified by Fall Farm Festivus. Patty Jones was a joy to have at Liddell, especially when my experimental design was geared towards self-torture. Her intelligence, clear thinking, and perspective made her the perfect person to ask whether I was going overboard (and even after I kept chugging along). Kevin Loope graciously welcomed a snot-nosed kid into the Seeley Lab. As an older academic brother, he was everything one could hope for: intelligent, hard working, and forgiving. I'm not the only one who thinks Kevin Loope should have been the blueprint for all humans on earth. David Peck and I shared an office for 6+ years, which in some states makes us legally married. He has a remarkable ability to translate gibberish into intelligent ideas. Callum Kingwell has a smile that could have saved the Titanic. Despite being clock-shifted, I am always impressed by his work ethic, knowledge of the literature, and good humor. Cissy Ballen is the reason that the Pollinator Garden is filled with zinnias and Sun Gold tomatoes. She showed infinite patience during the field season, or at least hid it well. From big disgusting breakfasts, to biking around lakes, Cissy initiates and creates fun around her. Her Sansa tree bore fruit after only two years. She keeps me on my toes.

The last and most sincere thank you goes to Tom, and his truck, Greenie. Tom and Greenie put up with more than everyone else combined. I never got Greenie stuck anywhere, and Tom certainly never had to rescue me with his tractor. Tom was the ideal mentor, friend, and colleague, right from the start. He's left big shoes to fill, but luckily, I wear flip-flops.

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CHAPTER 1

HONEY BEE SOCIOMETRY: TRACKING HONEY BEE COLONIES AND THEIR NEST CONTENTS FROM COLONY FOUNDING UNTIL DEATH

Michael L. Smith, Madeleine M. Ostwald, Thomas D. Seeley

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ABSTRACT

Sociometry is the description and analysis of the physical and numerical attributes of social insect colonies over their lifetimes. Sociometric data, such as worker number and nest size, are essential to understanding how colonies develop but are rarely collected. Even *Apis mellifera*, the most intensively studied social insect, has never received a broad-scale sociometric study. To help fill this gap, we monitored four honey bee colonies living in large observation hives from when they began as swarms (July 2012), to when they died (January 2014). We tracked multiple colony parameters, including worker and drone populations, comb area and use, swarming rate, and colony death. Each colony's life history is described through its founding, ergonomic, and reproductive stages.

INTRODUCTION

The pattern of growth and development that leads to a mature social insect colony is as impressive as its final form. To understand socio*genesis*, how a colony changes as it develops (Wilson 1985), we must conduct studies of socio*metry*, the

description and analysis of the physical and numerical attributes of social insect colonies (Tschinkel 1991). Without sociometric data, we cannot know what life-history strategies colonies follow, how they grow during their development, what resources they amass, and ultimately how they die. Most social insect biologists, however, have analyzed certain parts of a colony's functioning—such as food collection, communication, and reproduction—without first carefully describing the colonies that they are studying (Tschinkel 1991; Starr 2006). An exception is Tschinkel, who both proposed a list of sociometric data that can be collected from social insect colonies (Tschinkel 1991), and then did so, for multiple species of ants (e.g. *Solenopsis invicta*: Tschinkel 1993; *Pogonomyrmex badius*: Tschinkel 1998; *Pheidole morrisi*: Murdock and Tschinkel 2015).

Given how intensively honey bee colonies have been studied, it is surprising that we are still lacking a detailed sociometric study of *Apis mellifera*. Various studies have looked at a few parameters of established honey bee colonies such as brood area and worker number, but have left out other colony parameters, such as size of honey stores, number of reproductives, or area of drone comb (Nolan 1925; Bodenheimer and Ben-Nerya 1937; Farrar 1937; Allen 1958; Allen 1965; Winston 1979; Winston 1980a; Winston 1980b; Winston et al 1981; Lee and Winston 1987; McNally and Schneider 1992). A comprehensive study of honey bee colony growth and development, however, needs to start with incipient colonies (swarms). Two studies have tracked honey bee colonies from when they began, but one did not record the comb contents (Lee and Winston 1985), and the other did not record the worker and drone populations (Pratt 1999). Neither study tracked colonies year

round. The most complete study to date examined colonies that began as swarms and tracked each colony's comb building, comb contents, worker population, and drone population (Rangel and Seeley 2012). In this study, however, the investigators provided some comb to the colonies, and they tracked them only through their first winter, so they could not report on their second year, when colonies typically produce reproductives.

Perhaps no sociometric study can be truly comprehensive, but a broad-scale study must meet two criteria: (1) track the colony throughout its life, from when it is founded to when it dies, and (2) track as many colony parameters as possible, in concert. To do this, we tracked four honey bee colonies living in large observation hives from birth to death while recording worker population, drone population, comb area, comb use (cells holding brood, pollen, honey, or nothing), swarming and afterswarming events, and time of death.

METHODS

We set up four large observation hives, installed in each one a swarm of bees, and then observed the colonies as they grew, developed, and died. The study ran from July 2012 through January 2014 and was performed at the Liddell Field Station of Cornell University, in Ithaca, NY (42° 27.6' N, 76° 26.7' W).

Observation hives

Each colony's name indicates the direction that its hive entrance faced (North, East, and South Colonies, all in one building), or the room it occupied (Office

Colony, entrance faced north). Hives were built as described previously (Seeley 1995, Figure 4.2, p. 73), but were four times larger (North Colony: 0.95 x 1.0 m, East Colony, South Colony, and Office Colony: 0.88 x 1.0 m, as in Pratt 1999). Each one had a 4 x 4 cm grid drawn on its two glass walls. We used large observation hives so their volumes (North Colony: 41 liters; East, South and Office Colonies: 38 liters) were typical for a natural nesting cavity (45 L) (Seeley and Morse 1976). The hives were empty when the swarms were installed (i.e., were without frames containing beeswax comb) and we let the colonies build the amount, shape, and type of comb they desired.

Preparing and installing swarms and feeding colonies

On 2 July and 8 July 2012, we prepared artificial swarms to install in the observation hives (see Seeley and Tautz 2001 for methods). These swarms contained 11550 ± 889 bees, the size of natural swarms in Ithaca (Fell et al. 1977). We measured swarm size by weight (7700 bees in 1 kg: Mitchell 1970; Otis 1982). Each swarm contained a first-year, naturally mated queen reared at the lab. We fed the swarms 1:1 (vol:vol) sucrose solution ad libitum for 72-96 h before we installed them in the hives.

The North, South, and Office Colony swarms were installed on 6 July 2012; the East Colony swarm was installed on 10 July 2012. To install a swarm, we tilted back one of the glass walls of its hive, shook the bees onto the tilted glass so they slid into the hive, and then gingerly restored the glass wall to its vertical position. Swarms were installed relatively late for Ithaca, NY (most swarms occur 1 May to 15

July: Seeley and Visscher 1985), so to ensure that the colonies stored enough food to survive their first winter, we fed each one 0.5 L of 1:1 (vol:vol) sucrose solution about every other day from 13 July 2012 to 19 October 2012. The Office Colony required feeding to prevent starvation in the spring of 2013, so we provided 0.5 L of a 1:1 (vol:vol) sucrose solution on 12 March, 30 March, 16 April, and 20 April 2013. We also fed ca. 5 L of a 2:1 (vol water:vol sugar) sucrose solution to each colony between 25 September to 2 October 2013. These feedings were needed because our study colonies were within 100 m of 20 full-size colonies and so experienced greater than natural competition for forage (Seeley 2007)

Colony manipulations

During the first winter, the room housing the North, East, and South Colonies was kept above 7 °C, and the room housing the Office Colony was kept at 20°C. During the second winter, we shut off the heat in the room holding the North, East, and South Colonies, to see how they would handle colder temperatures. We treated the colonies for *Varroa destructor*, using Apistan® strips on 9 April 2013, and Api-Life VAR® on 6 September 2013. We could not sample bees and brood to assay mite levels, because doing so would have severely disturbed the colonies. The South Colony's queen died during the first winter, and the workers did not replace her, so we requeened the colony on 9 April 2013.

Measuring colony parameters

Once colonies were installed in observation hives, we repeatedly measured these variables: worker population, drone population, comb area, and comb use. Comb use (for worker comb and drone comb) was the area of cells holding either pollen, brood, honey, or nothing. We also recorded swarming, afterswarming, and colony death.

The worker population and drone population of each colony was measured once a month at night (when all bees were inside hive). To estimate each colony's worker and drone populations, we randomly selected 45 of the 4 x 4 cm grid squares containing bees, counted the workers and drones in each square, and multiplied the average number of workers and drones per square by the number of squares containing bees. We did not measure colony populations over winter, because we could not make accurate counts of the tightly clustered bees (November 2012 to March 2013 and September 2013 to January 2014).

We also estimated each colony's population when it began building drone comb, began producing drone brood, and began acquiring adult drones. To do this, we noted the colony's population on the two closest dates before and after the date of interest, calculated the slope of the line between these two dates, and estimated the colony's population on the date of interest by interpolation.

To track swarming and afterswarming events, we monitored the following cues: appearance of queen cells, break in brood cycle, sudden drop in worker population, replacement of marked queen, and swarm settling within 100 m of the hive. When possible, we caught the swarm, weighed it and a sample of 20+ bees,

and then estimated the swarm's population from the weight data. We estimated the number of drones by measuring the proportion of drones in 45 subsamples, and then multiplying that proportion by the swarm's population. We also measured the populations of workers and drones left in the observation hive after swarming, using methods described above. Knowing the number of bees in the swarm, and in the observation hive, we determined the size of the colony before it swarmed.

To track the development of each colony's nest, we measured its comb area and comb contents each week. For this, we placed a sheet of clear Mylar over each side of each observation hive. We then traced the area of worker comb and drone comb; drone comb was distinguished from worker comb by its larger cells; wall-to-wall dimension: drone cell, 6.2–6.9 mm; worker cell, 5.2–5.7mm (Martin and Lindauer 1966; Taber and Owens 1970). To measure the comb contents, we traced the comb areas containing pollen, honey, brood (eggs, larvae, or pupae), or nothing (empty cells). Next, we photographed each Mylar sheet with a size reference. Then we calculated, using Adobe Photoshop CS6 Version 13.0.1, the comb's total area and its area of cells for each type of contents. Separate measurements were made for worker comb and drone comb.

There were fluctuations in comb area measurements due to imprecision in the tracing technique. This explains, for example, the total comb area fluctuations during the winter weeks (Figure 1.1). Also, for one colony on one date (East Colony, 28 May 2013), we failed to trace one side of the hive. We assumed in this instance that the comb contents on the side that was not traced matched those on the side that was traced.

RESULTS AND DISCUSSION

We follow the colonies chronologically through their first and second years. Within each year, we subdivide by developmental stage, and then compare the colonies.

First Year

1. Founding stage

In most social insect species, colonies are started by a single queen that must rear the first workers herself, but in *Apis mellifera* colonies are started by a queen accompanied by 13300 ± 9600 workers (Lee and Winston 1985). In swarming species, the founding stage is defined as the time from when a nest site is inhabited to when the first workers eclose (Oster and Wilson 1978, p. 30)

The workers in each colony began building worker comb immediately after being installed in their hive in early July. A few days later, the queen in each colony began laying eggs in the new comb. By the end of July, the 4 colonies had built 57 ± 6% of the comb they would build in the first year (range 52–64%). Previous studies have also reported that colonies build comb quickly following colony founding; for example, Lee and Winston (1985) found that 90% of the comb built in the first year was built in the first 44 days. In all four colonies, the area of worker comb increased sharply, then nearly plateaued in early August, and then increased again until 17 September 2012, after which no more comb was built in 2012 (Figure 1.1). In the first year, the colonies built the majority (65 ± 22%, range 47–97%) of the comb that their nests would hold by the end of the second year (Figure 1.1).

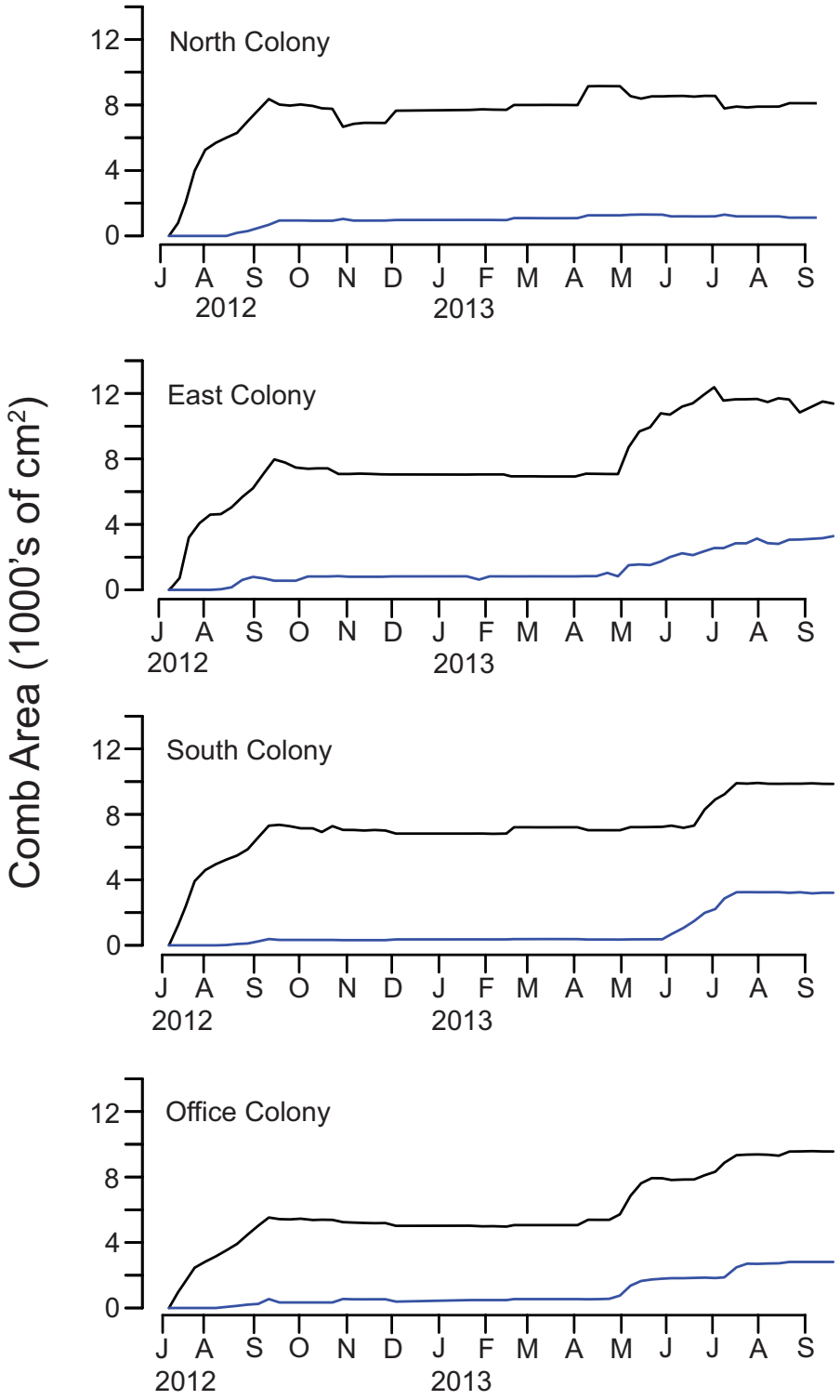

Figure 1.1 Construction of worker comb (upper black line) and drone comb (lower blue line) over time. Comb areas include both sides of each colony's comb

During the founding stage, each colony's worker population decreased because foragers were dying while no new workers were eclosing (Figure 1.2). The earliest the colonies could have new workers was 21 days post installation: 27 July 2012 for North, South, and Office Colonies, and 31 July 2012 for East Colony. Worker populations decreased by 68 ± 15% (range 56–90%) by the first censusing date, 26 July 2012. By the next censusing date, 30 August 2012, the worker population in all four colonies had begun to increase.

2. Ergonomic stage

Once workers begin to emerge, a colony transitions from founding stage to ergonomic stage and often starts growing rapidly (Oster and Wilson 1978, p. 34). The worker populations of all four colonies increased, but only the North Colony's population surpassed its founding size in the first year (11755 workers on 1 October 2012, Figure 1.2). All four colonies continued to build worker comb and rear worker brood during the ergonomic stage. Because the ergonomic stage ends when a colony starts investing in reproduction, the ergonomic stage lasted only 5-6 weeks (from early July to mid August) for all four colonies. After this, each colony began building drone comb; a honey bee colony's first investment in reproduction (reviewed by Boes 2010).

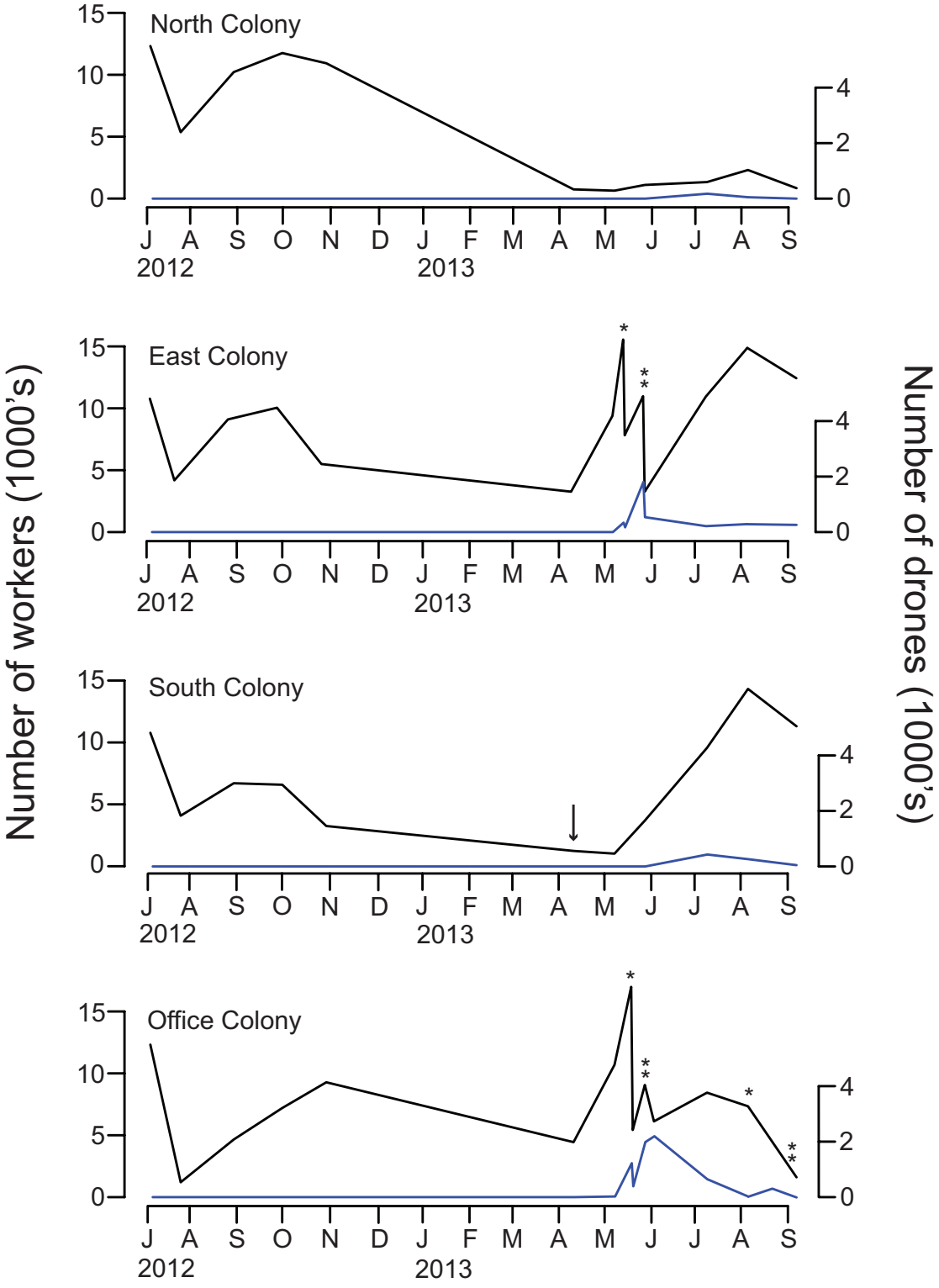

Figure 1.2 Number of workers (upper black line) and drones (lower blue line) for each of the four colonies. Swarming is marked by *, afterswarming by **. Arrow on South Colony marks when the colony was requeened.

3. Reproductive stage

The reproductive stage begins when a colony starts investing in reproduction (Oster and Wilson 1978, p. 50). Whereas a colony with an annual cycle usually makes a fairly discrete switch from producing workers to producing reproductives (Macevicz and Oster 1976), a perennial colony continues producing workers while producing reproductives. Therefore, a honey bee colony's investments in growth and reproduction overlap. All four colonies built drone comb in their first year, starting 39 ± 5 days after nest construction began (see Table 1.1), concurrent with building worker comb (Figure 1.1). The colonies began building drone comb when they had 5919 ± 1797 workers (range: 3671–7859). This is consistent with prior work, which found that honey bee colonies start building drone comb when they possess at least 4000 workers (Smith et al. 2014).

When comb building ceased in all four colonies in the first year (by 17 September 2012), drone comb made up 7 ± 2% of each colony's comb area (range: 4–11%). Even though all four colonies invested in reproduction in the first year by building drone comb, none of them began producing drones. Both building drone comb and producing drones are costly (Seeley 2002), and first-year colonies struggle to amass enough honey to survive their first winter, especially in northern locations like Ithaca, NY. Evidently, however, it pays to build drone comb because eventually (in the second year) this comb will be needed for rearing drones and in the meantime it can be used for storing honey (Smith et al 2015). Indeed, on 17 September 2012, 79 ± 23% of the drone comb was filled with honey (range: 51–100%).

4. First winter

A honey bee colony faces its greatest risk of mortality during its first winter; only 24% of feral colonies survive it in Ithaca, NY (Seeley 1978), probably because few colonies manage to stockpile the ca. 20 kg of honey needed to survive winter (Seeley and Visscher 1985). On 3 December 2012, the mean comb area filled with honey in the four colonies was 4220 ± 1109 cm^2 (range: 2878–5519). Given that 3.25 g of honey can be stored in 1 cm^2 of comb (Pratt 1999), and given that our colonies had 2110 ± 555 cm^2 of comb filled with honey, we estimate they had stored 6.9 ± 1.8 kg of honey. Because the colonies had little honey going into their first winter, we predicted that they would not survive it, but they did. Office Colony required feeding in the spring of 2013 to prevent starvation, but the other three colonies survived on their own. These three colonies, however, were housed in a heated room with a minimum temperature of 7 °C, so their metabolic costs of thermoregulation were lower than colonies exposed to normal winter temperatures (Southwick 1983).

Colonies also need at least 2000 workers in the spring to restart colony growth (Free and Racey 1968). On 30 October 2012, the last population count for 2012, there were 7239 ± 3498 workers in the colonies (range: 3250–10929). It seemed that all four colonies might have enough workers to restart colony growth in the following spring.

5. First year summary

When the four colonies started in July, they were essentially identical, but by

late October they were markedly different. North Colony was strongest, with the largest population (10929 workers) and the largest nest (8626 cm^2 of comb). North Colony also had the most drone comb (11% of nest), hence had made the largest investment in reproduction. The South and East Colonies ended the year markedly weaker than the North Colony, with far smaller populations (3250 and 5498 workers, respectively), smaller nests (7189 and 7873 cm^2 of comb), and less drone comb (5% and 10% of nest). The Office Colony was intermediate with a sizable population (9278 workers), but the smallest nest (5409 cm^2 of comb, 7% drone comb).

Table 1.1: Sociometrics of the four colonies

	North Colony	East Colony	South Colony	Office Colony	Mean ± SD
Date of installation	6 July 2012	10 July 2012	6 July 2012	6 July 2012	NA
# workers at installation	12320	10780	10780	12320	11550 ± 889
Age and date at first drone comb	38 days 13 Aug 2012	34 days 13 Aug 2012	38 days 13 Aug 2012	45 days 20 Aug 2012	39 ± 5 days
# workers at first drone comb	7859	6713	5431	3671	5919 ± 1797
Maximum proportion of drone comb	0.11	0.22	0.25	0.23	0.20 ± 0.06
Age and date at first drone brood	327 days 28 May 2013	287 days 23 Apr 2013	333 days 3 June 2013	291 days 18 Apr 2013	310 ± 24 days
# workers at first drone brood	1101*	6107	4665	6005	4470 ± 2340
Maximum proportion of drone comb used for drone brood and date	0.00* 28 May 2013	0.90 7 May 2013	0.60 11 June 2013	0.92 7 May 2013	0.61 ± 0.43
Maximum proportion of drone comb used for honey storage and date	0.58 3 Dec 2012	0.90 25 June 2013	0.95 16 July 2013	0.96 8 July 2013	0.85 ± 0.18
Maximum proportion of drone comb empty and date	1.00 30 July 2013	0.69 18 Sept 2013	1.00 20 Aug 2013	1.00 29 Jan 2013	0.92 ± 0.16
Age and date at first adult drones	368 days 8 July 2013	310 days 15 May 2013	368 days 8 July 2013	307 days 8 May 2013	338 ± 34 days
# workers at first adult drones	1334	15553	10952	10689	9632 ± 5966
Maximum # of workers and date	11755 1 Oct 2012	15553 15 May 2013	14324 4 Aug 2013	16988 19 May 2013	14655 ± 2219
Maximum # of drones (% of colony) and date	177 (12%) 8 July 2013	1809 (14%) 28 May 2013	430 (4%) 8 July 2013	2195 (26%) 3 June 2013	1153 ± 999 14 ± 9%
Age and date at first swarm; swarm size	Did not swarm	311 days 16 May 2013 7900 bees	Did not swarm	319 days 20 May 2013 12394 bees	315 ± 6 days 10147 ± 3178 bees
# workers before first swarm	N/A	15553	N/A	16988	16271 ± 1015
# primary swarms and date	0	1, 16 May 2013	0	2, 20 May 2013 and 3 Aug 2013	0.75 ± 0.96
# afterswarm(s), date, and size	0	1, 29 May 2013, 8991 bees	0	2, 3 June 2013, 1179 bees and 20 Aug 2013, 7084 bees	0.75 ± 0.96

Maximum total proportion of honey stores and date	0.53 3 Dec 2012	0.90 3 June 2013	0.81 23 July 2013	0.78 3 June 2013	0.76 ± 0.16
Maximum total proportion of worker brood and date	0.26 8 July 2013	0.53 14 May 2013	0.69 18 June 2013	0.68 14 May 2013	0.54 ± 0.20
Maximum total proportion of drone brood and date	0.00* 28 May 2013	0.13 7 May 2013	0.10 18 June 2013	0.16 14 May 2013	0.10 ± 0.07
Maximum total proportion of empty comb and date	0.99 13 Aug 2013	0.58 2 Apr 2013	0.86 20 Aug 2013	1.00 27 Aug 2013	0.86 ± 0.20
Maximum total proportion of pollen stores and date	0.04 14 May 2013	0.13 7 May 2013	0.06 7 May 2013	0.10 7 May 2013	0.08 ± 0.04
% worker mortality over first winter (# that died)	93% (10188)	41% (2234)	62% (2014)	52% (4834)	62 ± 22% (4818 ± 3802)
Age and date of death	428 days 6 Sept 2013	527 days 18 Dec 2013	572 days 28 Jan 2014	475 days 23 Oct 2013	501 ± 63 days
Apparent cause of death	Weak colony, unable to recover after first winter.	Exposure to cold. Still had food stores.	Exposure to cold. Still had food stores.	Failed to requeen on 25 Sept 2013.	NA

*A few drone eggs were laid in worker cells

SECOND YEAR

1. Emerging from winter

We measured the colonies' worker populations on 11 April 2013 and found that all four had shrunken over winter (see Table 1.1, Figure 1.2). Worker mortality averaged 62 ± 22% (range: 41–93%). The North Colony had only 741 workers, and the South Colony had only 1236 workers. The East Colony and Office Colony were stronger, with 3000+ workers in each (3264 and 4444 workers, respectively). High worker mortality over winter is normal, so colonies have their smallest populations in early spring (March, Avitabile 1978; April, this study), but we do not know why the North Colony lost 93% of its workers. The colony still had large honey stores, so the dead workers had not starved.

In late winter, from January-March 2013, three of the four colonies maintained a small brood nest (North: 82 ± 62 cm^2; East: 65 ± 53 cm^2; Office: 323 ± 234 cm^2). South Colony had no brood because its queen died over winter. The colony was otherwise healthy, so we requeened it on 9 April 2013 with a naturally mated, 1-year-old queen. The brood nests in the North and East Colonies during the winter consisted mainly of eggs. The Office Colony had three times more brood than the others, due perhaps to being housed in a warmer room (20°C minimum in office versus 7°C in the observation hive room). Indeed, the Office Colony was different from the other three colonies throughout the winter. It maintained more brood (Figure 1.3) and did not form a tight winter cluster. By early March, however, the Office Colony had consumed nearly all its honey stores, so we provided a sugar syrup feeder to prevent starvation. After 2 April 2013, brood production began to

increase in the East Colony and the Office Colony, followed by the South Colony on 23 April 2013 (Figure 1.3).

2. Ergonomic stage

The ergonomic stage began with surges in the colonies' brood rearing and worker populations (Figure 1.2 and 1.3). Workers began building additional worker comb when the area of empty worker comb dropped to 19 ± 6% (range 14–26%) (Figure 1.3). This finding matches the report of Pratt (1999), who found that workers start building comb when the area of empty comb has dropped to 20-25% and nectar is being gathered (1999). The pattern of ergonomic growth seen in the East, South, and Office Colonies was not seen in the North Colony. Its worker population did not rise steeply, it had large areas of empty comb, and it built no new comb (Figure 1.1-1.3).

3. Reproductive stage

The ergonomic stage was followed shortly by the reproductive stage. While building worker comb and rearing worker brood, three of the colonies also started building drone comb and rearing drones. At this point, the four colonies fell into three categories: 1) reproduced by producing drones and swarms (East and Office), 2) reproduced by producing only drones (South), and 3) did not reproduce (North).

3.1 Producing drones

The first sign of reproduction in the second year was the appearance of drone brood in mid-April in all the colonies, except the North Colony (Figure 1.3).

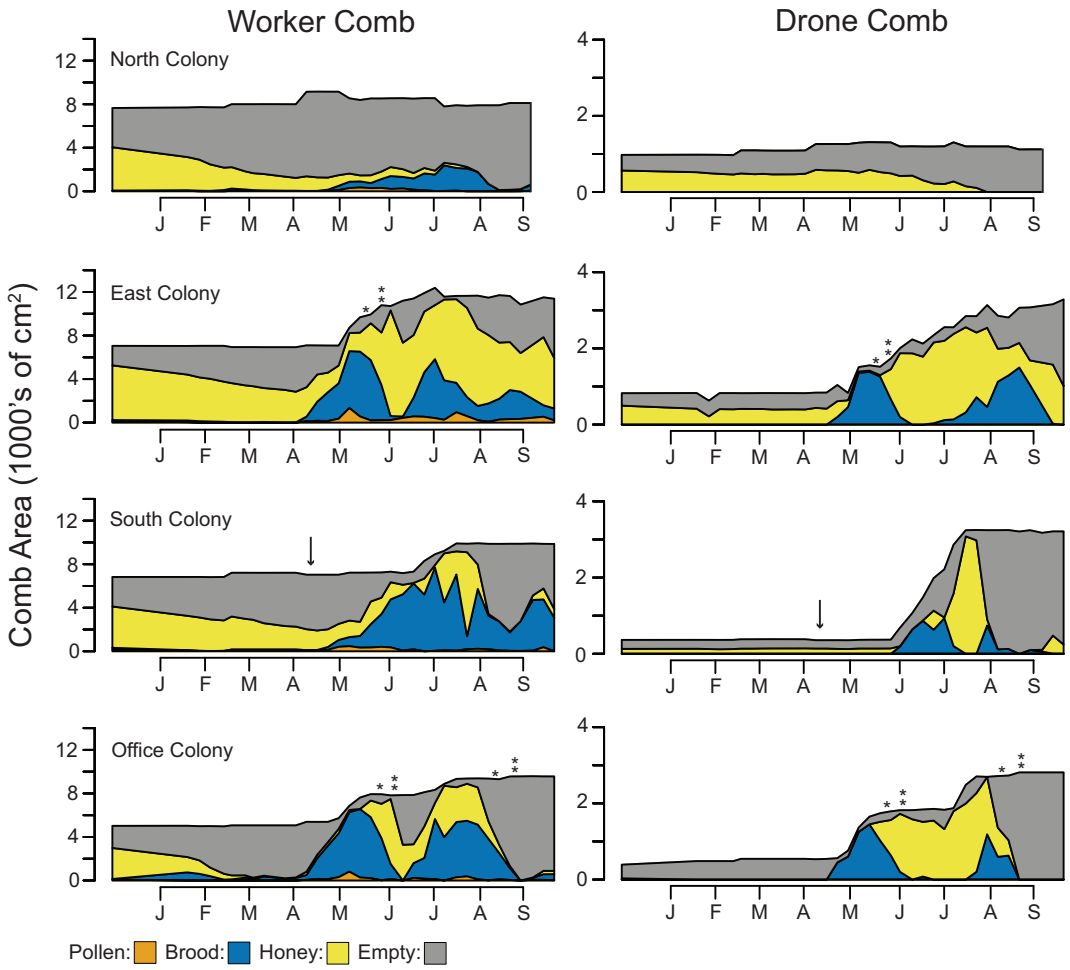

Figure 1.3 Comb contents for the four colonies, from 3 Dec 2012 to 18 Sept 2013. Worker comb (left) and drone comb (right). Swarming is marked by *, afterswarming by **. Arrows on plots for South Colony mark when the colony was requeened. North Colony was declared dead on 6 Sept 2013. Comb areas include both sides of each colony's comb.

Drone rearing began when the colonies had 5592 ± 805 workers (range: 4665–6107) (Table 1.1), a population size strikingly similar to what the colonies had when they began building drone comb in the first year (range: 3671–7859).

The first adult drones were seen in Office Colony on 8 May 2013, closely followed by East Colony on 15 May 2013 (see Table 1.1). Adult drones first appeared in South Colony on 8 July 2013. Excluding the North Colony (see alternative reproductive tactics), the colonies had 12398 ± 2735 workers when the first adult drones emerged (range: 10689–15553). The pattern of drone production in our study matches previous studies, which found that drone brood has two peaks: one before swarming season and another in late summer (Allen 1958; Lee and Winston 1987; this study, Figure 1.3).

Each colony, except North, also built additional drone comb (Figure 1.1). Indeed, the East, South, and Office Colonies built most of their drone comb in the second year, with the average area increasing from 410 ± 129 cm^2 in the first year, to 3103 ± 254 cm^2 in the second. Thus, 87 ± 4% of the drone comb was built in the second year. The area of worker comb, in contrast, increased only 33 ± 9% in the second year, from 6920 ± 430 cm^2 to 10267 ± 975 cm^2, for the same three colonies. Workers started building drone comb when the percent drone comb that was empty fell to 38 ± 29 percent (range 20–71%) (Figure 1.3). If workers use the same triggers for building drone comb and worker comb (comb building starts when there is little empty comb and much incoming nectar (Pratt 1999)), then workers appear more sensitive to a drop in area of empty comb for drone comb (threshold of

38 ± 29%) than for worker comb (threshold of 19 ± 6%). South Colony even built drone comb when still 71% of its drone comb was empty!

3.2 Producing swarms and afterswarms

Only the East and Office Colonies produced swarms and afterswarms (see Table 1.1). Both produced a prime swarm in mid-May, followed by afterswarms 13–14 days later. The Office Colony produced a second prime swarm in early August, followed by a second afterswarm 17 days later. The South Colony never swarmed, probably because it started its worker production late due to queen loss over winter (Figure 1.2). The North Colony also never swarmed, probably because it never rebuilt its worker population.

What induces swarming? To compare colony parameters between colonies that did and did not swarm, we calculated the population and comb contents for each colony on 14 May 2013, the last measurement date just before the East and Office Colonies swarmed (see Table 1.2). The colonies that produced swarms, relative to those that did not, had larger worker populations ($p = 0.005$) and larger areas of both worker brood ($p = 0.005$) and drone brood ($p = 0.030$). The areas of worker comb, drone comb, and total comb were not significantly different between colonies that produced swarms and those that did not ($p > 0.05$), nor were the areas of comb containing pollen, honey, or nothing ($p > 0.05$). The number of adult drones also did not differ between the two groups ($p > 0.05$). Large colony size is one of the four factors—large colony size, brood nest congestion, abundance of young workers, and reduced transmission of queen pheromones—that are hypothesized to stimulate swarming (reviewed by Grozinger et al. 2013). Our

results support the hypotheses that large colony population and abundance of worker brood stimulates swarming.

We also compared colony parameters between before swarming (14 May 2013), and before afterswarming (28 May 2013), to see how the colonies differed in these two contexts. Ideally, we would have compared colonies that swarmed and afterswarmed to colonies that swarmed but did not afterswarm, but both colonies in our study that swarmed also afterswarmed. During the 2-week interim period between swarming and afterswarming, the workers in both colonies continued to build worker comb and drone comb (see Table 1.3). They also boosted their colonies' honey stores, although not significantly, from 0.1% and 16% of the comb area to 42% and 45%. In Table 1.3, we see that before swarming colonies had significantly more workers ($p = 0.040$), a larger area of worker brood ($p = 0.036$) and a larger area of drone brood ($p = 0.032$), than they did before afterswarming. This is expected, because a colony that afterswarms has already cast workers in the primary swarm, and it lacks a laying queen.

Table 1.2: Colony parameters on 14 May 2013, before two of the colonies swarmed.

	Swarming Colonies		Non-Swarming Colonies		Significance
	East	Office	North	South	T test
Worker Population	15553	16988	777	1819	$t = 16.86$, $df = 1.83$, $^*p = 0.005$
Drone Population	340	1224	0	0	$t = 1.77$, $df = 1.00$, $p = 0.328$
Worker Comb Area (cm^2)	9677	7618	8388	7220	$t = 0.71$, $df = 1.58$, $p = 0.566$
Drone Comb Area (cm^2)	1554	1650	1308	367	$t = 1.62$, $df = 1.02$, $p = 0.349$
Total Comb Area (cm^2)	11231	9268	9696	7587	$t = 1.12$, $df = 1.99$, $p = 0.381$
Pollen Stores	5%	3%	3%	4%	$t = 0.86$, $df = 1.00$, $p = 0.546$
Honey Stores	16%	0.1%	12%	19%	$t = 0.44$, $df = 1.05$, $p = 0.733$
Empty Comb	14%	13%	79%	3%	$t = 0.69$, $df = 1.01$, $p = 0.616$
Worker Brood	53%	68%	6%	14%	$t = 17.85$, $df = 1.83$, $^*p = 0.005$
Drone Brood	12%	16%	0%	0%	$t = 20.94$, $df = 1.00$, $^*p = 0.030$

Significance tests for pollen stores, honey stores, empty comb, worker brood, and drone brood were calculated based on nest area, not percentage.

Table 1.3: Colony parameters of the two colonies before swarming (14 May 2013) and before afterswarming (28 May 2013)

	Before Swarming		Before Afterswarming		Significance
	East	Office	East	Office	T test
Worker Population	15553	16988	10967	9063	$t = 5.25$, $df = 1.86$, $^*p = 0.040$
Drone Population	340	1224	1809	1985	$t = 2.47$, $df = 1.08$, $p = 0.230$
Worker Comb Area (cm^2)	9677	7618	10785	7924	$t = 0.40$, $df = 1.82$, $p = 0.731$
Drone Comb Area (cm^2)	1554	1650	1735	1791	$t = 2.90$, $df = 1.61$, $p = 0.130$
Total Comb Area (cm^2)	11231	9268	12520	9715	$t = 0.51$, $df = 1.79$, $p = 0.668$
Pollen Stores	5%	3%	2%	0%	$t = 1.56$, $df = 1.79$, $p = 0.262$
Honey Stores	16%	0.1%	45%	42%	$t = 3.34$, $df = 1.96$, $p = 0.081$
Empty Comb	14%	13%	22%	11%	$t = 0.61$, $df = 1.10$, $p = 0.646$
Worker Brood	53%	68%	26%	40%	$t = 7.09$, $df = 1.56$, $^*p = 0.036$
Drone Brood	12%	16%	5%	7%	$t = 10.47$, $df = 1.31$, $^*p = 0.032$

Significance tests for pollen stores, honey stores, empty comb, worker brood, and drone brood were calculated based on nest area, not percentage.

What were the fates of the swarms and afterswarms produced in May and June? A swarm that moves into a tree cavity in Ithaca has a probability of 0.24 of surviving the following winter (Seeley 1978), though the number of workers in a swarm strongly influences this probability. Rangel and Seeley (2012) report winter survival probabilities as a function of swarm size: 0.78 for large swarms (10K+ bees), 0.22 for medium swarms (5-10K bees), and 0.00 for small swarms (less than 5K bees). Assuming swarms and afterswarms have the same relationship between swarm size and survival probability, the East Colony produced a swarm and an afterswarm that each had a 0.22 probability of survival, and the Office Colony produced a swarm that had a 0.78 probability of survival and an afterswarm that had a 0.00 probability of survival. On 3 August 2013, Office Colony swarmed and afterswarmed again, which is perplexing because a swarm cast in August is exceedingly unlikely to amass large enough honey stores to survive winter.

3.3 Drones in swarms

Drones were not distributed equally between swarm and the colony left in the original hive. The 3 June 2013 afterswarm from the Office Colony contained 10% drones (123 drones and 1056 workers). The colony fraction still in the observation hive, however, contained 26% drones (2195 drones, 6121 workers). Therefore, only 5% of the 2138 drones in the colony left in the afterswarm. Likewise, the 20 August 2013 afterswarm from the Office Colony contained 5% drones, whereas the colony fraction in the observation hive contained 33% drones. We noticed the same pattern in primary swarms, but unfortunately only collected

the data reported. These data raise questions about how and why only a small minority of the drones, but the large majority of the workers (ca. 75%; Rangel and Seeley 2012), leave in a swarm or afterswarm. Drones may have better survival, and thus reproductive success, if they stay at home. Those that depart with a swarm are a drain on the colony as it builds its new nest, so they may face a higher likelihood of eviction than if they stay in the parental nest, which is well stocked with beeswax combs and honey stores.

Staying at home, however, is not without risks. When the Office Colony cast an afterswarm on 20 August 2013, the proportion of drones left in the observation hive was extremely high (33% drones). Two days later, when we measured the worker and drone populations, we found a 4-cm layer of dead drones on the hive's floor, but no dead workers. We do not know how the workers culled their drone population, but simply withholding food from the drones would not work because drones can feed themselves from honey cells (Free 1957). We do, however, know that this colony was low on honey stores, and that colonies reduce their investment in drone brood when forage is limited (Seeley and Mikheyev 2003). In this case, workers reduced their drone investment by killing adults, not brood (the colony had no drone brood). When drone eviction occurs in the fall, adult drones are driven out of the hive and left to starve (Free 1957). The drones that we saw, however, died within the observation hive. Perhaps the drone-to-worker ratio was too high to evict the drones, but the workers were nevertheless able to kill the drones. How workers decide they must cull their adult drones awaits future research.

3.4 Alternative reproductive tactics

The North Colony reared a few drones from queen-laid eggs in July 2013 but, curiously, these drones were reared in worker comb instead of drone comb. The drones reared in worker cells were approximately two-thirds smaller in total body size than drones produced in drone cells in our other colonies. Smaller drones are capable of mating with queens, but they produce fewer sperm (Schluns et al. 2003), and have lower reproductive success than larger drones (Berg et al. 1997). Evidently, the production of these small drones was a final effort by the dying North Colony to gain genetic success.

4. Second winter

By 5 September 2013, it was obvious that the North and Office Colonies were unlikely to survive their second winter. The North Colony had only 840 workers and 43 cm^2 (0.14 kg) of honey stores. This colony had never recovered from its high worker loss the previous winter. The queen laid numerous eggs, but the workers were too few to rear all the larvae from these eggs. The North Colony was unable to increase its worker population over the summer, so it was unable to collect honey stores. The Office Colony was also weak at the end of its second summer. It had only 1604 workers, and 28 cm^2 (0.09 kg) of honey stores. Unlike the North Colony, the Office Colony was strong in the spring and cast a swarm in May and an afterswarm in June. Moreover, this colony swarmed and afterswarmed again in August, but then it failed to rebuild its worker population and amass large honey stores, hence it was deathly weak going into the second winter.

By contrast, the East and South Colonies were strong going into the second winter. On 5 September 2013, the East Colony had 12,483 workers and 4718 cm^2 (15.33 kg) of honey stores, and the South Colony had 11304 workers and 455 cm^2 (1.48 kg) of honey stores. Although the South Colony did not have as much honey stored as the East Colony, the worker force was strong with the South Colony, and it acquired much honey from goldenrod (*Solidago* spp.) in September.

5. Colony Death

By September, it was clear that the North and Office Colonies were dying; they had few workers, no brood, and meager honey stores. The North Colony was dead on 6 September 2013 and by the end of September the Office Colony was essentially dead. This colony had a new laying queen on 27 August 2013, and it had resumed brood production on 4 September 2013, but on 20 September 2013 it attempted to replace its queen and failed to do so. Without a laying queen, this colony's population gradually declined until the last workers died on 23 October 2013.

The East Colony, which was the strongest colony going into the winter, was found dead on 18 December 2013, evidently from exposure to cold. The minimum temperature on 18 December 2013 was -8°C, and the minimum temperature for the two days prior was -16°C. The colony still had honey stores, so it did not starve. We had treated all the colonies for *Varroa* mites, and we know that colonies housed in small cavities have lower *Varroa* infestation rates (Loftus et al. 2016), so we are confident that none died due to *Varroa* and associated viruses. Similarly, the South

Colony was found dead on 28 January 2014. We presume that the cause of death for this colony was also exposure to cold. The minimum temperature on 21 January 2014 was -24°C, which dropped ever further to -27°C the following day. This colony still had honey stores and, like the East Colony, it had been treated for mites. During the first winter, we had maintained the observation hive room at 7°C. In the second winter, however, we decided to let the temperature fluctuate naturally. This was a mistake. A colony living in an observation hive is spread over a single comb and this prevents a colony from forming a thick, well-insulated winter cluster. If we had again kept the observation hive room at 7°C, as in the first winter, then probably both East and South Colonies would have survived their second winter.

6. Caveats

Sociometric studies can always be improved and expanded. For example, we initiated our colonies with artificial swarms, but it would be better to have used natural swarms. Also, future work would benefit from a larger sample size. We kept colonies in observation hives, but future studies could allow the bees to build their nests in hives with empty movable frames. This would enable researchers to gather data that we were unable to collect, such as *Varroa* mite levels, and colony mass. Parallel combs in a cavity would also help overwintering by enabling the bees to form a normal winter cluster. Collecting data weekly, however, would be disruptive to the colony, and potentially destructive, especially during the winter.

7. Final Remarks

This broad-scale sociometric study tracked multiple colony parameters from when each colony first occupied its nest site to when it died. We observed different life-history trajectories that colonies used to maximize their genetic success, depending on colony state. A colony that is strong, with numerous workers after the first winter, is able to cast swarms and produce drones in its second year, and simultaneously amass honey stores to survive their second winter. A colony that is weak after the first winter can regain strength and also reproduce in its second year, but perhaps only by producing drones, and in some cases, only small drones.

We have reported our findings on an individual colony basis, so readers can see similarities and differences among the colonies. While we expected variation among them, we did not expect colonies to shift between strength and weakness as quickly as they did. For example, the North Colony never recovered after high worker mortality in the first winter, and the Office Colony swarmed itself to death. Colony strength is surprisingly ephemeral.

KEY WORDS

Growth and development, tracking developmental changes, colony morphology, lifetime reproductive investment, social insects

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REFERENCES

Allen MD (1958) Drone brood in honey bee colonies. J Econ Entomol 51:46–48. doi: http://dx.doi.org/10.1093/jee/51.1.46

Allen MD (1965) The production of queen cups and queen cells in relation to the general development of honeybee colonies, and its connection with swarming and supersedure. J Apic Res 4:121–141. doi: 10.1080/00218839.1965.11100115

Avitabile A (1978) Brood rearing in honeybee colonies from late autumn to early spring. J Apic Res 17:69–73. doi: 10.1080/00218839.1978.11099905

Berg S, Koeniger N, Koeniger G, Fuchs S (1997) Body size and reproductive success of drones (*Apis mellifera* L). Apidologie 28:449–460. doi: 10.1051/apido:19970611

Bodenheimer FS, Ben-Nerya A (1937) One year studies on the biology of the honey-bee in Palestine. Ann Appl Biol 24:385–403. doi: 10.1111/j.1744-7348.1937.tb05041.x

Boes KE (2010) Honeybee colony drone production and maintenance in accordance with environmental factors: an interplay of queen and worker decisions. Insectes Soc 57:1–9. doi: 10.1007/s00040-009-0046-9

Farrar CL (1937) The influence of colony populations on honey production. J Agric Res 54:945–954.

Fell RD, Ambrose JT, Burgett DM, et al (1977) Seasonal cycle of swarming in honeybees. J Apic Res 16:170–173. doi: 10.1080/00218839.1977.11099883

Free JB (1957) The food of adult drone honeybees (*Apis mellifera*). Brit J Anim Behav 1:14–18. doi: 10.1016/S0950-5601(57)80038-0

Free JB, Racey PA (1968) The effect of the size of honeybee colonies on food consumption, brood rearing and the longevity of the bees during winter. Entomol Exp Appl 11:241–249. doi: 10.1007/BF00305232

Grozinger CM, Richards J, Mattila HR (2013) From molecules to societies: mechanisms regulating swarming behavior in honey bees (*Apis* spp.). Apidologie 45:327–346. doi: 10.1007/s13592-013-0253-2

Lee PC, Winston ML (1985) The effect of swarm size and date of issue on comb construction in newly founded colonies of honeybees (*Apis mellifera* L.). Can J Zool 63:524–527. doi: 10.1139/z85-077

Lee PC, Winston ML (1987) Effects of reproductive timing and colony size on the survival, offspring colony size and drone production in the honey bee (*Apis mellifera*). Ecol Entomol 12:187–195. doi: 10.1111/j.1365-2311.1987.tb00997.x

Loftus JC, Smith ML, Seeley TD (2016) How honey bee colonies survive in the wild: testing the importance of small nests and frequent swarming. PLoS One 11:e0150362. doi: 10.1371/journal.pone.0150362

Macevicz S, Oster G (1976) Modeling social insect populations II: optimal reproductive strategies in annual eusocial insect colonies. Behav Ecol Sociobiol 1:265–282. doi: 10.1007/BF00300068

Martin H, Lindauer M (1966) Sinnesphysiologische Leistungen beim Wabenbau der Honigbiene. Z Vergl Physiol 53:372–404. doi: 10.1007/BF00298103

McNally LC, Schneider SS (1992) Seasonal cycles of growth, development and movement of the African honey bee, *Apis mellifera scutellata*, in Africa. Insectes Soc 39:167–179. doi: 10.1007/BF01249292

Mitchell C (1970) Weights of workers and drones. Am Bee J 110:468–469.

Murdock TC, Tschinkel WR (2015) The life history and seasonal cycle of the ant, *Pheidole morrisi* Forel, as revealed by wax casting. Insectes Soc 62:265–280. doi: 10.1007/s00040-015-0403-9

Nolan WJ (1925) The brood-rearing cycle of the honeybee. United States Dep Agric Dep Bull 1349:1–56.

Oster GF, Wilson EO (1978) Caste and Ecology in the Social Insects. Princeton University Press

Otis GW (1982) Weights of worker honeybees in swarms. J Apic Res 21:88–92. doi: 10.1080/00218839.1982.11100520

Pratt SC (1999) Optimal timing of comb construction by honeybee (*Apis mellifera*) colonies: a dynamic programming model and experimental tests. Behav Ecol Sociobiol 46:30–42. doi: 10.1007/s002650050589

Rangel J, Seeley TD (2012) Colony fissioning in honey bees: size and significance of the swarm fraction. Insectes Soc 59:453–462. doi: 10.1007/s00040-012-0239-5

Schluns H, Schluns EA, Praagh J van, Moritz RFA (2003) Sperm numbers in drone honeybees (*Apis mellifera*) depend on body size. Apidologie 34:577–584. doi: 10.1051/apido

Seeley TD (1995) The Wisdom of the Hive. Harvard University Press, Cambridge, MA

Seeley TD, Visscher P (1985) Survival of honeybees in cold climates: the critical timing of colony growth and reproduction. Ecol Entomol 10:81–88. doi: 10.1111/j.1365-2311.1985.tb00537.x

Seeley TD (2002) The effect of drone comb on a honey bee colony's production of honey. Apidologie 33:75–86. doi: 10.1051/apido

Seeley TD (1978) Life history strategy of the honey bee, *Apis mellifera*. Oecologia 32:109–118. doi: 10.1007/BF00344695

Seeley TD, Mikheyev AS (2003) Reproductive decisions by honey bee colonies: tuning investment in male production in relation to success in energy acquisition. Insectes Soc 50:134–138. doi: 10.1007/s00040-003-0638-8

Seeley TD, Morse RA (1976) The nest of the honey bee (*Apis mellifera* L.). Insectes Soc 23:495–512. doi: 10.1007/BF02223477

Seeley TD, Tautz J (2001) Worker piping in honey bee swarms and its role in preparing for liftoff. J Comp Physiol A 187:667–676. doi: 10.1007/s00359-001-0243-0

Smith ML, Ostwald MM, Loftus JC, Seeley TD (2014) A critical number of workers in a honeybee colony triggers investment in reproduction. Naturwissenschaften 101:783–790. doi: 10.1007/s00114-014-1215-x

Smith ML, Ostwald MM, Seeley TD (2015) Adaptive tuning of an extended phenotype: honeybees seasonally shift their honey storage to optimize male production. Anim Behav 103:29–33. doi: 10.1016/j.anbehav.2015.01.035

Southwick EE (1983) The honey bee cluster as a homeothermic superorganism. Comp Biochem Physiol Part A Physiol 75:641–645. doi: 10.1016/0300-9629(83)90434-6

Starr CK (2006) Steps toward a general theory of the colony cycle in social insects. In: Kipyatkov VE (ed) Life cycles Soc. insects Behav. Ecol. Evol. St. Petersburg University Press, St. Petersburg, pp 1–20

Taber S, Owens CD (1970) Colony founding and initial nest design of honey bees *Apis mellifera* L. Anim Behav 18:625–632. doi: 10.1016/0003-3472(70)90005-9

Tschinkel WR (1991) Insect sociometry, a field in search of data. Insectes Soc 38:77–82. doi: 10.1007/BF01242715

Tschinkel WR (1998) Sociometry and sociogenesis of colonies of the harvester ant, *Pogonomyrmex badius*: worker characteristics in relation to colony size and season. Insectes Soc 45:385–410. doi: 10.1007/s000400050097

Tschinkel WR (1993) Sociometry and sociogenesis of colonies of the fire ant *Solenopsis invicta* during one annual cycle. Ecol Soc Am 63:425–457. doi: 10.2307/2937154

Wilson EO (1985) The sociogenesis of insect colonies. Science 228:1489–1495.

Winston ML (1980a) Seasonal patterns of brood rearing and worker longevity in colonies of the Africanized honey bee (Hymenoptera: Apidae) in South America. J Kansas Entomol Soc 53:157–165.

Winston ML (1980b) Swarming, afterswarming, and reproductive rate of unmanaged honeybee colonies (*Apis mellifera*). Insectes Soc 27:391–398. doi: 10.1007/BF02223731

Winston ML (1979) Intra-colony demography and reproductive rate of the Africanized honeybee in South America. Behav Ecol Sociobiol 4:279–292. doi: 10.1007/BF00297648

Winston ML, Dropkin JA, Taylor OR (1981) Demography and life-history characteristics of two honey bee races (*Apis mellifera*). Oecologia 48:407–413. doi: 10.1007/BF00346502

CHAPTER 2

A CRITICAL NUMBER OF WORKERS IN A HONEY BEE COLONY TRIGGERS INVESTMENT IN REPRODUCTION

Michael L. Smith, Madeleine M. Ostwald, J. Carter Loftus, Thomas D. Seeley

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ABSTRACT

Social insect colonies, like organisms, must decide as they develop how to allocate optimally their resources among survival, growth, and reproduction. Only when colonies reach a certain state do they switch from investing purely in survival and growth to investing also in reproduction. But how do worker bees within a colony detect that their colony has reached the state where it is adaptive to begin investing in reproduction? Previous work has shown that larger honey bee colonies invest more in reproduction (i.e. the production of drones and queens), however, the term 'larger' encompasses multiple colony parameters including number of adult workers, size of the nest, amount of brood, and size of the honey stores. These colony parameters were independently increased in this study to test which one(s) would increase a colony's investment in reproduction via males. This was assayed by measuring the construction of drone comb, the special type of comb in which drones are reared. Only an increase in the number of workers stimulated construction of drone comb. Colonies with over 4000 workers began building drone comb, independent of the other colony parameters. These results show that

attaining a critical number of workers is the key parameter for honey bee colonies to start to shift resources towards reproduction. These findings are relevant to other social systems in which a group's members must adjust their behavior as a function of the group's size.

INTRODUCTION

As an organism grows and develops, its investment in reproduction increases (Kozlowski 1992). Just as a single organism grows, develops, and eventually reproduces, a colony of social insects, made up of many individuals, must coordinate its developmental transitions (reviewed by Starr 2006). The mechanisms controlling the timing of these developmental transitions are well studied in certain unicellular and multicellular organisms (yeast- reviewed by Turner et al. 2012; bacteria- reviewed by Chien et al. 2012; *Caenorhabditis elegans* and *Drosophila melanogaster*- reviewed by Thummel 2001), but we know little about the mechanisms that control these transitions in units at the next higher level of biological organization, such as colonies of social insects.

How has a social insect colony changed when it starts to invest in reproduction, and how do the members of the colony recognize this change? In bumble bee colonies (*Bombus spp*.), the number of reproductives produced is correlated with the number of workers in the colony (Webb 1961, cited in Michener 1964). The onset of colony reproduction, however, is independent of worker number (Pomeroy and Plowright 1982), and appears to be initiated by the queen reaching a certain age. Colonies with older queens start their reproduction earlier

than those with younger queens, controlling for the number and age of workers (Alaux et al. 2005). Furthermore, experimentally removing workers from a colony does not change the onset of reproduction in bumble bee colonies (Muller and Schmid-Hempel 1992). In colonies of certain species of ants, reproduction is evidently connected to the number and nutritional state of the workers. Larger *Myrmica spp.* colonies produce more reproductive males than smaller colonies, and reproductive females are produced only when colonies contain over 900 workers (Brian 1957). Large colonies of fire ants (*Solenopsis invicta*) invest a steady proportion of resources into reproductives, but they do so only after surpassing a population threshold somewhere between 20000 and 50000 ants, at which point there is also a substantial increase in body fat in workers (Tschinkel 1993). In colonies of the harvester ant, *Pogonomyrmex occidentalis*, greater colony size increases the probability of reproduction, but not the number of reproductives (Cole and Wiernasz 2000).

In all these examples in which a social insect colony's investment in reproduction is a function of its size, it is unknown whether the increased investment in reproduction is directly linked to the number of workers in the colony or to some related parameter. What parameters do the workers monitor to decide whether their colony should begin reproduction? Colony parameters such as nest size, number of workers, amount of brood, and level of food stores, all change as a colony develops (Wilson 1985; Tschinkel 1993; Tschinkel 1998), but they are rarely studied (Tschinkel 1991; Tschinkel 2011). Descriptions of parameters associated with the onset of reproduction cannot reveal what specific parameters the workers

monitor, because many of the parameters change in parallel as a colony develops. Here, we present a controlled experiment designed to identify which colony parameter(s) triggers workers to start investing in male reproduction in honey bee colonies.

A honey bee colony is a protandrous hermaphrodite; it invests in male reproductives before it invests in female reproductives (Page 1981). The first stage of rearing male reproductives – hereafter, drones – is building drone comb, the special comb made of large cells in which drones are reared (reviewed by Boes 2010). Therefore, the start of drone comb construction is the first sign of reproductive investment by a honey bee colony. Previous research has shown that larger colonies build more drone comb, and probably start doing so sooner (Free and Williams 1975; Lee and Winston 1985; Henderson 1991; Rangel and Seeley 2012). However, larger colonies are different from smaller colonies in many ways; they have more workers, more worker brood, more comb, and greater stores of food (honey and pollen) (Rangel and Seeley 2012). We manipulated the following four colony parameters to determine which one or ones, affects a colony's construction of drone comb: number of workers, area of worker comb, amount of brood, and size of honey stores. For each parameter, we hypothesized that if reaching a critical level of this variable is the trigger for starting to invest in male reproduction, then increasing this parameter in a colony should result in a colony producing more drone comb. These four hypotheses were separately tested to see which colony parameter, when boosted, leads to an increased investment in drone comb.

METHODS

Definitions

Frame: wooden structure in a movable-frame hive that holds a beeswax comb. Langstroth standard frames, used in this experiment, measure 48 x 23 cm. An "empty frame" contains no comb; a "comb frame" contains comb with empty cells; a "honey frame" contains comb filled with honey; and a "brood frame" contains comb filled with brood.

Brood: collective term for eggs, larvae, and pupae.

Drone comb: comb used for rearing drones. Drone comb is easily identified because the cells of drone comb are larger than those of worker comb (wall-to-wall distance: 6.2-6.9 mm vs. 5.2-5.7 mm) (Martin and Lindauer 1966; Taber and Owens 1970).

Package: a box made of wood and wire screen (15cm x 25cm x 35cm) that contains several thousand worker bees and one queen bee. The bees in a package are fed by brushing sucrose solution on the screen sides.

Final colony population size: the number of adult worker bees in a colony at the end of the experiment.

General setup

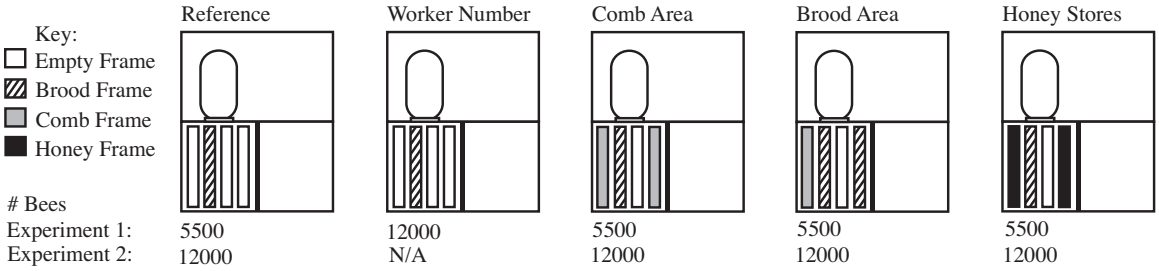
All experiments were performed at the Liddell Field Station of Cornell University, in Ithaca, NY (42° 27.6' N, 76° 26.7' W).

The general plan of the study was to establish groups of colonies that differed in just one parameter, and to see which (if any) differences between the groups influenced the amount of drone comb built (see Figure 2.1). Each group began with

11 (Experiment 1) or 8 (Experiment 2) colonies. Within each group, each colony was installed in a 10-frame hive that contained empty frames within which the workers in the colony could build comb. The underside of the top bar of each empty frame was coated with a layer of beeswax to encourage comb building in line with the other combs. To ensure that the colonies always had ample space for building new comb, we supplied each colony with an additional empty frame each time we inspected it. We inspected each colony three times over 14 days, and each time we measured the area of newly built comb, making separate measurements for drone comb and worker comb.

The experiments tested the four hypotheses about what colony parameter fosters the building of drone comb: worker number, comb area, brood area, and honey stores. To promote comb building in general, we gave each colony a feeder filled with a 50:50 (vol:vol) sucrose solution. Hive volume was kept the same for all treatments; wooden partitions separated the occupied and empty portions of each hive. Colonies that were given fewer frames containing comb (comb frames, brood frames, or honey frames) were given more empty frames, so all colonies occupied the same amount of space. The wooden partitions were moved to accommodate the empty frames added on the observation dates. The experimental setup at the start of both experiments is illustrated in Figure 2.1.

Figure 2.1 The five treatments. Each hive consisted of two hive boxes. The top hive box held a feeder, and the bottom hive box held the test frames (initially four, as shown). The type of frame is indicated by the pattern. The thick black line in the lower hive box represents the movable wooden partition for adjusting the volume of each hive.



Hypotheses, predictions, and tests

H1: Worker number hypothesis: predicts that colonies that have more workers will build more drone comb. Tested by comparing the worker number treatment to the reference treatment.

H2: Comb area hypothesis: predicts that colonies that have more worker comb will build more drone comb. Tested by comparing the comb area treatment to the reference treatment.

H3: Brood area hypothesis: predicts that colonies that have more worker brood will build more drone comb. Tested by comparing the brood area treatment to the comb area treatment (to control for the total amount of comb provided in the two treatments).

H4: Honey stores hypothesis: predicts that colonies that have more honey stored will build more drone comb. Tested by comparing the honey stores treatment to the comb area treatment (again, to control for the total amount of comb provided in the two treatments).

Experiment 1 vs. Experiment 2

In Experiment 1, the baseline number of workers was 5500, but the worker number treatment had 12000 bees. In Experiment 2, the number of workers was increased to 12000 for all treatments. This was done to further test the comb area, brood area, and honey stores hypotheses using larger colonies. If colonies need a threshold number of workers before they build drone comb, and 5500 workers is below the threshold, then the results of Experiment 1 would not reveal the effects (if

any) of having additional comb area, brood area, or honey stores. In Experiment 2, the same comparisons were made for each hypothesis as above, but with more workers in each colony. If number of workers is the only parameter that influences the building of drone comb, then all colonies in Experiment 2 should build similar amounts of drone comb.

Experimental setup was the same for both experiments. The same queens were used for both experiments, and were randomly assigned to a treatment. Queen ID and the start date for each experiment were added as random variables in the generalized linear mixed model, but neither variable significantly improved the model. Experiment 1 began on 27 May 2013, Experiment 2 on 21 June 2013. We tested for seasonal effects, but found no significant differences between May and June. This is not surprising, because drone comb is a permanent structure, which is used for rearing drone brood in subsequent years.

Preparing and installing colonies

All colonies were started as packages, so that the number of worker bees per colony was controlled (see methodology for making packages in Seeley and Tautz 2001, referred to there as "artificial swarms"). Packages containing 5500 worker bees weighed 0.71 ± 0.03 kg, and packages containing 12000 worker bees weighed 1.52 ± 0.05 kg. There are ~7700 worker bees in 1 kilogram (Mitchell 1970; Otis 1982). Workers bees were collected from the Liddell Field Station apiaries. Each colony was headed by a new queen bee, purchased in May 2013 from C.F. Koehnen

and Sons, Ord Bend, California. The bees in the packages were fed 50:50 (vol:vol) sucrose solution *ad libitum* for 72-96 h before being installed in their hives.

On day 0, the packages were installed. Packages were assigned at random to the treatment groups, except in Experiment 1 where the packages with 12000 bees were assigned to the worker number treatment. All frames containing comb that were provided to the colonies (comb frames, brood frames, and honey frames) had no drone comb. Brood frames were collected from the Liddell Field Station apiaries and were immediately installed in the experimental hives with the packages to minimize brood mortality. Brood frames were ones in which at least 75% of the cells contained a larva or a pupa. Comb frames were completely empty of honey, pollen, and brood. Honey frames were completely full of honey.

After installation, colonies were left undisturbed except when the comb built in the empty frames was measured (days 3, 8 and 14 for Experiment 1; days 3, 7, and 12 for Experiment 2). The areas of built worker comb and drone comb were measured using a 2 x 2 cm grid. At the end of the observation period, we measured each colony's final colony population size. The number of workers was estimated using the Liebefeld method (Imdorf et al. 1987) on day 16 for Experiment 1 and on day 12 for Experiment 2,.

Data analysis

All statistical analyses were performed using R software version 2.15.1 and the packages lme4, lsmeans, and agricolae (R Core Team 2012). Colonies in which the queen stopped laying eggs were removed from the analysis. The mean

proportion of drone comb built over the observation period was calculated for each treatment and compared using a Kruskal-Wallis multiple comparison test with a Holm-Bonferroni correction. The data analysis focused on the proportion drone comb, rather than the area, to account for differences between treatments in the level of comb building. Only the *a priori* comparisons were used to test each hypothesis (see above). This analysis was used for both Experiment 1 and 2.

The data from Experiment 1 and 2 were pooled and used in a generalized linear mixed model. Colony number was set as a random variable. Treatment and sampling were analyzed as factors. Package size, final colony population size, and date were analyzed as integers. The best-fit model was determined by comparing AIC values with a Chi-Squared test (Akaike 1974). The best-fit binomial model included six explanatory variables: treatment, sampling, the interaction between treatment and sampling, date, final colony population size, and initial colony population size.

RESULTS

Drone comb construction

Every colony built comb, but not every colony built drone comb. Table 2.1 shows the mean amounts of comb built for the different treatment groups in terms of the total comb area, the drone comb area, and the proportion drone comb. In Experiment 1, only the reference treatment and the worker number treatment showed a significant difference in the proportion of drone comb built ($p = 0.0003$), with the colonies in the worker number treatment building a significantly higher

proportion of drone comb (see Table 2.2). In Experiment 2, in which all treatment groups started out with the same large number of workers, the proportions of drone comb built did not significantly differ between treatments (see Table 2.2). Each of the *a priori* pairs tests a competing hypothesis, visualized in Figure 2.2 and 2.3 as paired boxplots.

Table 2.1 Comb building by treatment group in Experiment 1 and 2. Values reported as mean ± sd.

Treatment	Experiment 1 or 2	# of colonies	Total Comb Area (cm^2)	Drone Comb Area (cm^2)	Proportion Drone Comb
Reference	1	10	1844 ± 242	8 ± 18	0.00 ± 0.01
Worker Number	1	10	3310 ± 384	278 ± 186	0.08 ± 0.05
Comb Area	1	8	1059 ± 260	26 ± 44	0.02 ± 0.04
Brood Area	1	9	1269 ± 327	63 ± 55	0.05 ± 0.04
Honey Stores	1	11	1100 ± 286	14 ± 28	0.01 ± 0.02
Reference	2	7	2641 ± 973	111 ± 154	0.03 ± 0.05
Comb Area	2	8	1926 ± 421	107 ± 158	0.05 ± 0.07
Brood Area	2	8	1888 ± 446	149 ± 134	0.07 ± 0.05
Honey Stores	2	7	1923 ± 534	138 ± 72	0.08 ± 0.04

Table 2.2 Results of statistical testing for differences in the proportion of drone comb built, and total comb built, between treatment groups in Experiment 1 and 2.

Hypothesis	Comparison	Experiment	Significance: Proportion of Drone Comb	Significance: Total Comb Built
H1	Reference - Worker Number	1	0.0003	0.005
H2	Reference - Comb Area	1	0.97	< 0.00001
H3	Comb Area - Brood Area	1	0.24	0.22
H4	Comb Area - Honey Stores	1	1.00	0.52
H2	Reference - Comb Area	2	1.00	0.47
H3	Comb Area - Brood Area	2	0.95	1.00
H4	Comb Area - Honey Stores	2	0.77	1.00

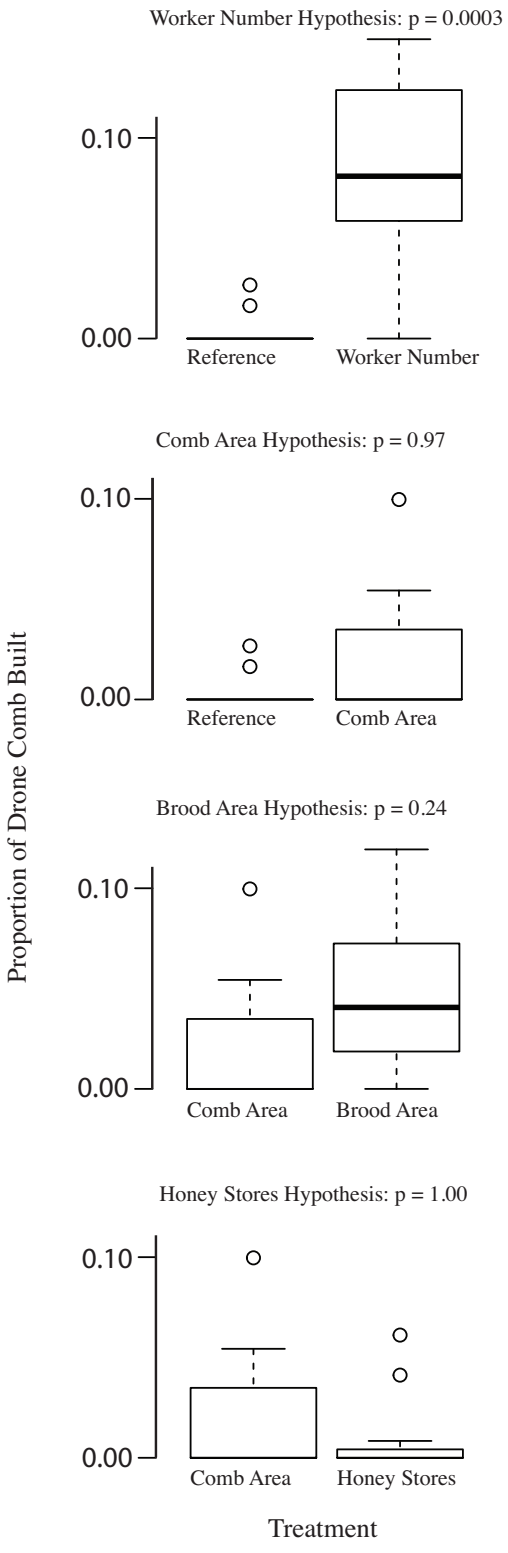


Figure 2.2 The proportion of drone comb built by colonies in Experiment 1. Boxplots show the proportions of drone comb built in each treatment group.

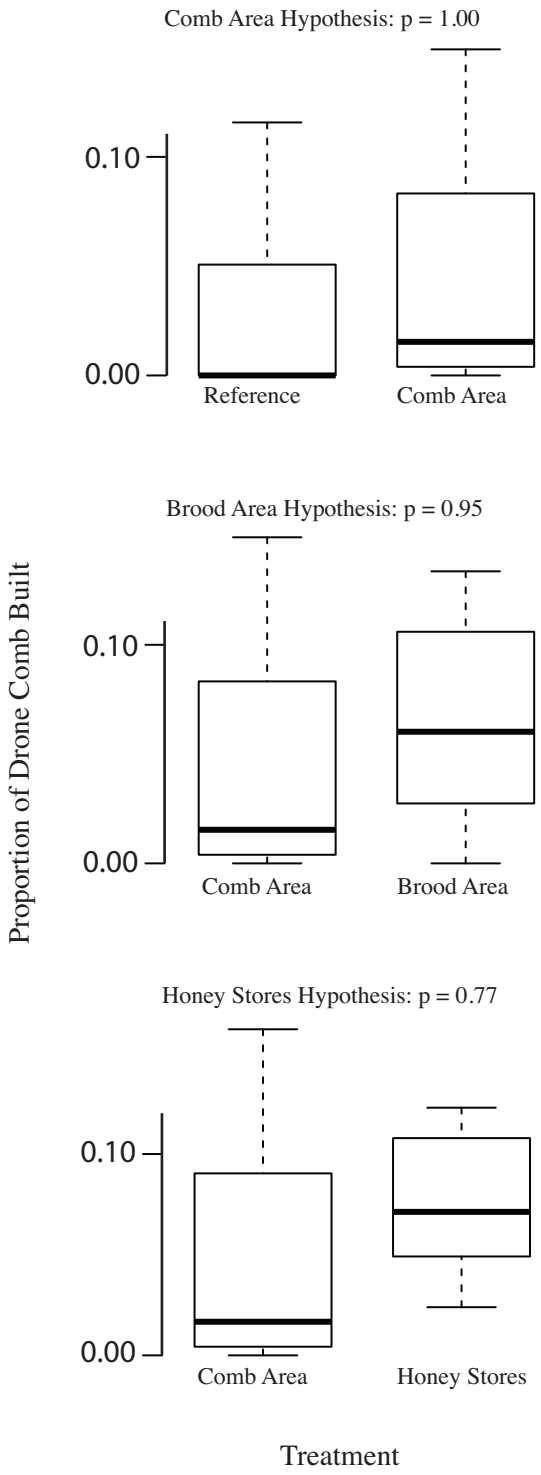


Figure 2.3 The proportion of drone comb built by colonies in Experiment 2, in which all colonies have 12000 workers. Boxplots show the proportions of drone comb built in each treatment group.

Model combining Experiment 1 and 2

The generalized linear mixed model that best explained the proportion of drone comb built (best fit determined by AIC, see methods), included treatment, sampling, the interaction between treatment and sampling, date, the final colony population size, and the package size. Colony was the random variable. The model has 78 groups, 234 observations, AIC= 975.4, and logLikelihood= -473.7. Of the explanatory variables, the only significant variable for explaining differences in the proportion of drone comb built was the final colony population size (estimate = $4.429e^{-04}$, standard error = $1.331e^{-04}$, p = 0.0009).

Across all colonies, as the final colony population size increased, the proportion of drone comb built increased (Figure 2.4). Colonies that had fewer than 3000 bees at the end of the experiment built almost no drone comb during the experiment. With one exception, all colonies with over 4500 bees at the end of the experiment built drone comb.

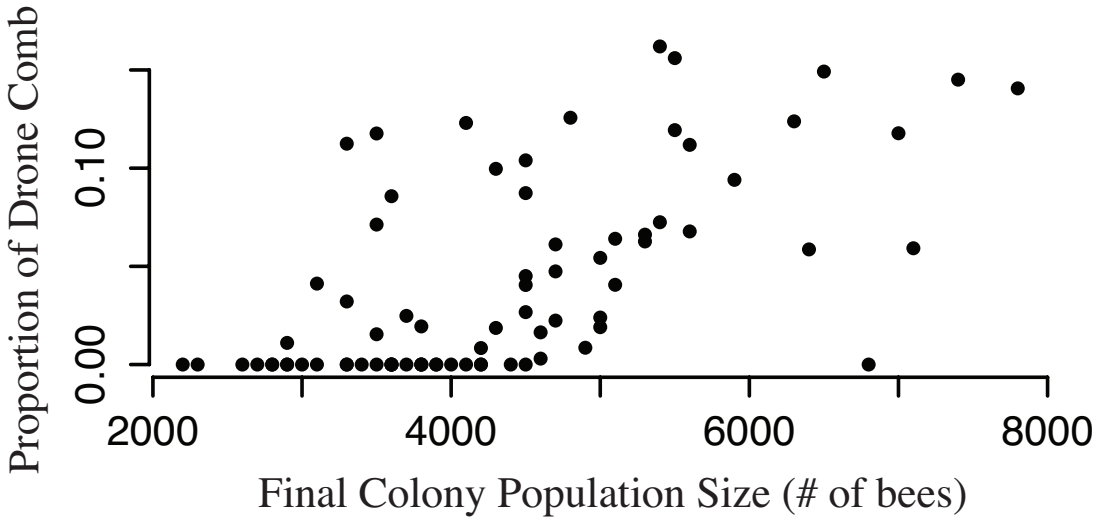


Figure 2.4 The proportion of drone comb built vs. the number of adult bees in the colony at the end of the experiment. There is a significant correlation between final colony population size and the proportion of drone comb built ($p = 0.0005$). Each point represents a single colony. Data points include all treatment groups from both experiments.

Total comb construction

The results of our experiments also shed light on how the total amount of comb built by a colony changes depending on colony parameters. We tested for differences in the total comb area built between the *a priori* pairs using a Kruskal-Wallis multiple comparison test (see Table 2.2). In Experiment 1, the worker number treatment built more total comb than the reference treatment ($p = 0.005$), and the reference treatment built more total comb than the comb area treatment ($p < 0.00001$). In Experiment 2, none of the *a priori* pairs showed a significant difference in total comb area.

DISCUSSION

The two experiments reported here tested which colony parameter(s) induces a developing colony of honey bees to begin investing in male reproduction by building drone comb. Evidently, the critical colony parameter for shifting a colony towards building drone comb is the number of workers. Colonies with more workers built a greater proportion of drone comb, but colonies with more comb, more brood, or more honey stores, did not do so. These results support the worker number hypothesis, which predicts that colonies use worker number, or a stimulus that varies with worker number, to determine when to begin investing in reproduction.

The five treatment groups were designed to create controlled tests of the four parameters of interest, but we did not have perfectly controlled tests because: (1) comb contents changed during the experiment, (2) adult bees eclosed from

brood frames, and (3) adult bees died while foraging. For example, colonies in the comb area treatment used their extra frames of comb for egg laying and nectar storage, which increased their brood areas and honey stores. Likewise, colonies in the brood area treatment had bees eclosing from their extra frames of brood, which increased the worker number in these colonies. And, as is shown in Figure 2.4, most colonies had fewer workers at the end of the experiment than at the start, probably from losing foragers. Despite this erosion in the differences between colonies in the different treatment groups, in Experiment 1 only the colonies in the worker number treatment built a significantly higher proportion of drone comb. The general linear mixed model using the results from both experiments also found that only final colony population size was significantly associated with drone comb building.

In Experiment 1, the 12000-bee treatment (worker number treatment), built significantly more comb overall than the 5500-bee treatment (reference treatment). This result is expected; a colony with more bees can build more comb. Furthermore, the 5500-bee treatment that received one wax comb (reference treatment) built significantly more comb overall than the 5500-bee treatment that received three wax combs (comb area treatment). While this result does not affect our conclusions about what triggers drone comb building, because these conclusions are based on the proportion of drone comb built, it does suggest that a shortage of empty comb stimulates comb construction, consistent with previous studies (Pratt 1999). The colonies in the comb area treatment had large areas of empty comb, so they had little need for more comb, hence it is not surprising that they built relatively little

comb. In Experiment 2, in which all colonies had 12000 workers, the colonies in the reference treatment (one comb) and those in the comb area treatment (three combs) did not build significantly different amounts of comb overall, perhaps because the increased number of workers were able to quickly fill the empty comb, thereby creating a need for more comb.

From Figure 2.4, we can estimate that a colony needs approximately 4000 workers to begin to invest in building drone comb. This is probably an underestimate, however, because the colonies' final population sizes were measured at the end of the experiment rather than when the colonies began building their drone comb.

These results raise an exciting question: how do individual worker bees acquire information about the number of workers in their colony? Research on nest building in the social wasp *Polybia occidentalis* suggests that workers in this species also "know" their colony's size and adjust their building accordingly, but the mechanisms remain unknown (Jeanne and Bouwma 2002). Experiment 2 may provide a clue for how this works with honey bees. In this experiment, colonies in the honey stores treatment had the highest mean proportion of drone comb built, and while inspecting the colonies in this treatment, we noticed that their workers did not cluster as tightly on their honey frames as they did on their comb frames or brood frames. If the workers did indeed cluster less tightly on their honey frames, then they may have had more bees clustered on the empty frames, where comb was being constructed. If worker congestion is used to measure the number of workers in a colony, this could explain the slight increase in drone comb building in colonies

in the honey stores treatment. Contact rates have been shown to regulate the allocation of workers among tasks in colonies of harvester ants (Pinter-Wollman et al. 2013), and honey bees (Seeley 1989). An increased number of workers in a colony, which may increase the worker contact rate, may also be a stimulus for swarming, the female mode of reproduction in honey bees (reviewed by Grozinger et al. 2013). But it remains unknown whether contact rates are used to assess the number of workers in a colony, and it is entirely possible that workers regulate their contact rates (Gordon et al. 1993) rather than being regulated by them.

The results from these experiments indicate that honey bees monitor the number of workers in the colony, or some related stimulus, to decide when to start investing in male reproduction by building drone comb. The onset of investment in reproduction is a critical switch point in the sociogenesis of social insect colonies (Wilson 1985). Exactly how the workers in a honey bee colony sense their number, to decide when to begin investing some of their colony's resources in reproduction, is a tantalizing mystery that beckons for further study.

KEYWORDS

sociogenesis, reproductive investment, reproductive timing, worker number, honey bees, drone comb

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REFERENCES

Akaike H (1974) A new look at the statistical model identification. Autom Control IEEETrans 19:716 – 723. doi: 10.1109/TAC.1974.1100705

Alaux C, Jaisson P, Hefetz A (2005) Reproductive decision-making in semelparous colonies of the bumblebee *Bombus terrestris*. Behav Ecol Sociobiol 59:270–277. doi: 10.1007/s00265-005-0035-6

Boes KE (2010) Honeybee colony drone production and maintenance in accordance with environmental factors: an interplay of queen and worker decisions. Insectes Soc 57:1–9. doi: 10.1007/s00040-009-0046-9

Brian MV (1957) The growth and development of colonies of the ant *myrmica*. Insectes Soc 4:177–190

Chien AC, Hill NS, Levin PA (2012) Cell size control in bacteria. Curr Biol 22:R340–R349. doi: 10.1016/j.cub.2012.02.032

Cole BJ, Wiernasz DC (2000) Colony size and reproduction in the western harvester ant, *Pogonomyrmex occidentalis*. Insectes Soc 47:249–255. doi: 10.1007/PL00001711

Free J, Williams I (1975) Factors determining the rearing and rejection of drones by the honeybee colony. Anim Behav 23:650–675

Gordon DM, Paul RE, Thorpe K (1993) What is the function of encounter patterns in ant colonies? Anim Behav 45:1083–1100. doi: 10.1006/anbe.1993.1134

Grozinger CM, Richards J, Mattila HR (2013) From molecules to societies: mechanisms regulating swarming behavior in honey bees (*Apis* spp.). Apidologie 45:327–346. doi: 10.1007/s13592-013-0253-2

Henderson CE (1991) Reproductive investment in drones in honey bee (*Apis mellifera*) colonies. Dissertation, Cornell University

Imdorf A, Buehlmann G, Gerig L, et al. (1987) Überprüfung der Schätzmethode zur Ermittlung der Brutfläche und der Anzahl Arbeiterinnen in freifliegenden Bienenvölkern. Apidologie 18:137–146

Jeanne RL, Bouwma AM (2002) Scaling in nests of a social wasp: a property of the social group. Biol Bull 202:289–295

Kozlowski J (1992) Optimal allocation of resources to growth and reproduction: implications for age and size at maturity. Trends Ecol Evol 7:15–19. doi: 10.1016/0169-5347(92)90192-E

Lee P, Winston ML (1985) The effect of swarm size and date of issue on comb construction in newly founded colonies of honeybees (*Apis mellifera* L.). Can J Zool 63:524–527

Martin H, Lindauer M (1966) Sinnesphysiologische Leistungen beim Wabenbau der Honigbiene. Z Vergl Physiol 53:372–404

Michener CD (1964) Reproductive efficiency in relation to colony size in hymenopterous societies. Insectes Soc 11:317–342

Mitchell C (1970) Weights of workers and drones. Am Bee J 110:468–469

Muller CB, Schmid-Hempel P (1992) Variation in life-history pattern in relation to worker mortality in the bumble-bee, *Bombus lucorum*. Funct Ecol 6:48–56

Otis GW (1982) Weights of worker honeybees in swarms. J Apic Res 21:88–92

Page RE (1981) Protandrous reproduction in honey bees. Environ Entomol 10:359–362

Pinter-Wollman N, Bala A, Merrell A, et al. (2013) Harvester ants use interactions to regulate forager activation and availability. Anim Behav 86:197–207. doi: 10.1016/j.anbehav.2013.05.012

Pomeroy N, Plowright R (1982) The relation between worker numbers and the production of males and queens in the bumble bee *Bombus perplexus*. Can J Zool 60:954–957

Pratt SC (1999) Optimal timing of comb construction by honeybee (*Apis mellifera*) colonies: a dynamic programming model and experimental tests. Behav Ecol Sociobiol 46:30–42. doi: 10.1007/s002650050589

R Core Team (2012) R: A language and environment for statistical computing

Rangel J, Seeley TD (2012) Colony fissioning in honey bees: size and significance of the swarm fraction. Insectes Soc 59:453–462. doi: 10.1007/s00040-012-0239-5

Seeley TD (1989) The honey bee colony as a superorganism. Am Sci 77:546–553

Seeley TD, Tautz J (2001) Worker piping in honey bee swarms and its role in preparing for liftoff. J Comp Physiol A 187:667–676. doi: 10.1007/s00359-001-0243-0

Starr CK (2006) Steps toward a general theory of the colony cycle in social insects.

In: Kipyatkov VE (ed) Life cycles Soc. insects Behav. Ecol. Evol. St. Petersburg University Press, St. Petersburg, pp 1–20

Taber S, Owens CD (1970) Colony founding and initial nest design of honey bees, *Apis mellifera* L. Anim Behav 18:625–632

Thummel CS (2001) Molecular mechanisms of developmental timing in *C. elegans* and *Drosophila*. Dev Cell 1:453–465

Tschinkel WR (2011) Back to basics: sociometry and sociogenesis of ant societies (Hymenoptera: Formicidae). Myrmecological News 14:49–54.

Tschinkel WR (1998) Sociometry and sociogenesis of colonies of the harvester ant, *Pogonomyrmex badius*: worker characteristics in relation to colony size and season. Insectes Soc 45:385–410. doi: 10.1007/s000400050097

Tschinkel WR (1991) Insect sociometry, a field in search of data. Insectes Soc 38:77–82. doi: 10.1007/BF01242715

Tschinkel WR (1993) Sociometry and sociogenesis of colonies of the fire ant *Solenopsis invicta* during one annual cycle. Ecol Soc Am 63:425–457

Turner JJ, Ewald JC, Skotheim JM (2012) Cell size control in yeast. Curr Biol 22:R350–R359. doi: 10.1016/j.cub.2012.02.041

Webb M (1961) The biology of the bumblebees of a limited area in eastern Nebraska. Dissertation, University of Nebraska

Wilson EO (1985) The sociogenesis of insect colonies. Science 228:1489–1495

CHAPTER 3

THE CUES OF COLONY SIZE: HOW HONEY BEES SENSE THAT THEIR COLONY IS LARGE ENOUGH TO BEGIN TO INVEST IN REPRODUCTION

Michael L. Smith, Phoebe A. Koenig, Jacob M. Peters

ABSTRACT

As organisms develop, they first invest resources in survival and growth, but after reaching a certain condition they start to also invest in reproduction. Likewise, superorganisms, such as honey bee colonies, first invest in survival and growth, and later commit resources to reproduction once the number of workers in the colony surpasses a reproductive threshold. The first form of reproductive investment for a honey bee colony is the building of beeswax comb made of special large cells used for rearing males (drones). How do the workers sense that their colony is large enough to start building this "drone comb"? To address this question, we experimentally increased three possible cues of colony size—worker density, volatile pheromone concentration, and nest temperature—and looked for effects on the bees' comb construction. Only the colonies that experienced increased worker density were stimulated to build a higher proportion of drone comb. We then monitored and quantified potential cues in small and large colonies, to determine which cues change with colony size. We found that workers in large colonies, relative to small ones, have increased contact rates, spend more time active, and experience less variable worker density. Whereas unicellular and multicellular

organisms use mainly chemical cues to sense their sizes, our results suggest that at least one superorganism, a honey bee colony, uses physical cues to sense its size and thus its developmental state.

INTRODUCTION

In virtually all living systems, developmental changes are cued to increases in the system's size. The bacteria *Vibrio fischeri*, which live in the light organ of the Hawaiian bobtail squid, *Euprymna scolopes*, begin emitting light only after reaching a high cell density (reviewed in Waters & Bassler 2005). Similarly, the cells of humans undergo changes associated with puberty only when body size has reached a threshold level (reviewed in Grumbach 2002). In both these examples, individual cells must detect group size, and then make appropriate changes. *Vibrio fisheri* use small hormone-like molecules to detect cell density, which act as transcription factors to alter gene expression (Waters & Bassler 2005). In humans, adipose tissue produces the hormone leptin, which must reach a critical level before puberty begins (Grumbach 2002). For unicellular bacteria and multicellular humans, the cues that trigger these developmental changes are chemical. Colonies of social insects that work together to form a tightly integrated unit (a "superorganism", Hölldobler & Wilson 2009), also have developmental changes linked to increases in group size. One of the most striking is the switch to investing in reproduction, not just survival and growth, once the number of workers in the colony exceeds a threshold level (Smith et al. 2014).

A honey bee colony reproduces by producing drones and casting swarms. A colony's *first* investment in reproduction, however, occurs when workers begin to build cells of beeswax comb with the large diameters needed for rearing drones, i.e. drone comb (reviewed in Boes 2010). Building drone comb marks the onset of colony "puberty", that is, the period during which a colony first begins to prepare for reproduction. Workers begin building drone comb once the number of workers in the colony has passed a reproductive threshold (Smith et al. 2014). In this paper, we use both experimental and observational approaches to address a key life-history question: how do the worker bees sense that their colony is large enough to begin to invest in reproduction?

Workers switch from building only worker comb to building both worker comb and drone comb when the colony has grown to have approximately 4000 workers (Smith et al. 2014). To detect this reproductive threshold, workers likely sense cues that are correlated with their colony's size rather than count the number of colony members per se. We used two complementary approaches to understand how individual bees sense colony size. First, we independently increased three possible cues of colony size: worker density, volatile pheromone concentration, and nest temperature. To assess whether bees respond to these cues by redirecting resources to reproduction, we measured the proportion of drone comb built after we experimentally increased each cue. Second, we quantified and compared potential cues in small and large colonies to determine which cues are reliable indicators of colony size.

MATERIALS AND METHODS

This research was performed at the Liddell Field Station of Cornell University, in Ithaca, NY (42°27.6′ N, 76°26.7′ W).

Experimental Study

In the experimental study we manipulated three cues that workers might use to sense that their colony has enough workers to invest in drone comb: (1) worker density, (2) volatile pheromone level, and (3) nest temperature. We chose these potential cues because of their relevance to honey bee life-history. Colonies living in the wild choose nest cavities of ca. 45 liters (Seeley & Morse 1976), so as the number of workers increases, so too may their density. Chemical cues are pervasive throughout honey bee communication (e.g. colony defense, brood presence, and queen status, reviewed in Slessor et al. 2005), so bees may use volatile pheromones to detect colony size. Finally, honey bees tightly regulate nest temperature (Seeley 1985, p.170), so increasing the number of heat-emitting individuals may change temperature gradients.

In each treatment group, we increased one of the three cues, while keeping colony size the same for each group. The size of each colony was sufficient to stimulate comb construction, but close to the threshold for building drone comb. We then compared the proportion of drone comb built by colonies in each treatment group to the control group. We reasoned that if the colonies in a treatment group built a higher proportion of drone comb than those in the control group, then this was evidence that workers in the treatment group use the manipulated cue as an indicator of colony size.

1. Hive Setup

The setup for each colony was as follows: a lower box, where the treatment was produced; a middle box, where the colony was kept; and an upper box, where the 2-liter 1:1 (vol:vol) sucrose feeder was placed to encourage comb building (Figure 3.1). A modified floorboard between the middle and lower boxes provided an entrance for the bees in the middle box. This floorboard also had two 7-cm holes with screen on both sides. The screen kept the bees in the middle box from entering the lower box, and the holes allowed the treatment in the lower box to permeate the middle box. Two Thermochron iButton Devices (model number DS1923FS from Embedded Data Systems; hereafter referred to as iButtons) measured temperature every 30 min at the center and the edge of each colony's middle box. The center iButton was placed in the center of the middle frame. The other was placed at the same height, but in the corner furthest from the hive entrance.

In the control treatment and density treatment, we left empty the lower box in each hive. In the density treatment, we increased the density of bees in the middle box by placing a wooden block in it, thus restricting the bees to 3 frames instead of 5 frames. Therefore, the bees in this treatment had less space in which to build comb, but having less space to build comb does not induce a colony to invest in drone comb (see Reference versus Comb Area in Figs 1-3 in Smith et al. 2014).

In the pheromone treatment, we installed a second colony in the lower box. This colony contained 10000 workers, five frames of brood with no drone comb, and a naturally mated queen. We installed colonies in the lower boxes two days

before the experimental colonies. To reduce mixing of the two colonies' foragers, we oriented the hive entrances of the two colonies in opposite directions.

In the temperature treatment, we installed a 40-watt incandescent lamp covered in tin foil in the lower box of each hive. A piece of wood placed above the lamp diffused the heated air in the lower box to prevent a hotspot from forming directly above the lamp. To check that each lamp stayed lit, we checked every 24 hours the temperature of each hive's lower box using a thermocouple thermometer (type K, Omega Engineering, Stamford, CT, USA).

Honey bees regulate the brood nest temperature between 33 and 36°C to ensure healthy development of the brood (Seeley 1985, p.170). To check that the lamp increased brood nest temperature but did not overheat the colony above, we monitored control hives with lit and unlit lamps, but without bees. The lit lamp increased the temperature of the middle box, but did not overheat it (heated control: 31.1° ± 7.2°C, unheated control: 24.4° ± 8.1°C). When we compared the brood nest temperatures of the colonies in the different treatment groups, we found that only the colonies in the temperature treatment had elevated brood nest temperatures. Relative to the control treatment colonies, the temperature treatment colonies were warmer ($P < 0.05$) at both center and edge of the middle box (temperature treatment: center 36.0° ± 1.8°C, edge 30.7° ± 5.8°C; control treatment: center 34.8° ± 1.3°C, edge 27.9° ± 5.5°C; density treatment: center 34.4° ± 2.4°C, edge 27.6° ± 4.7°C; pheromone treatment: center 34.8° ± 1.3°C, edge 28.8° ± 5.5°C).

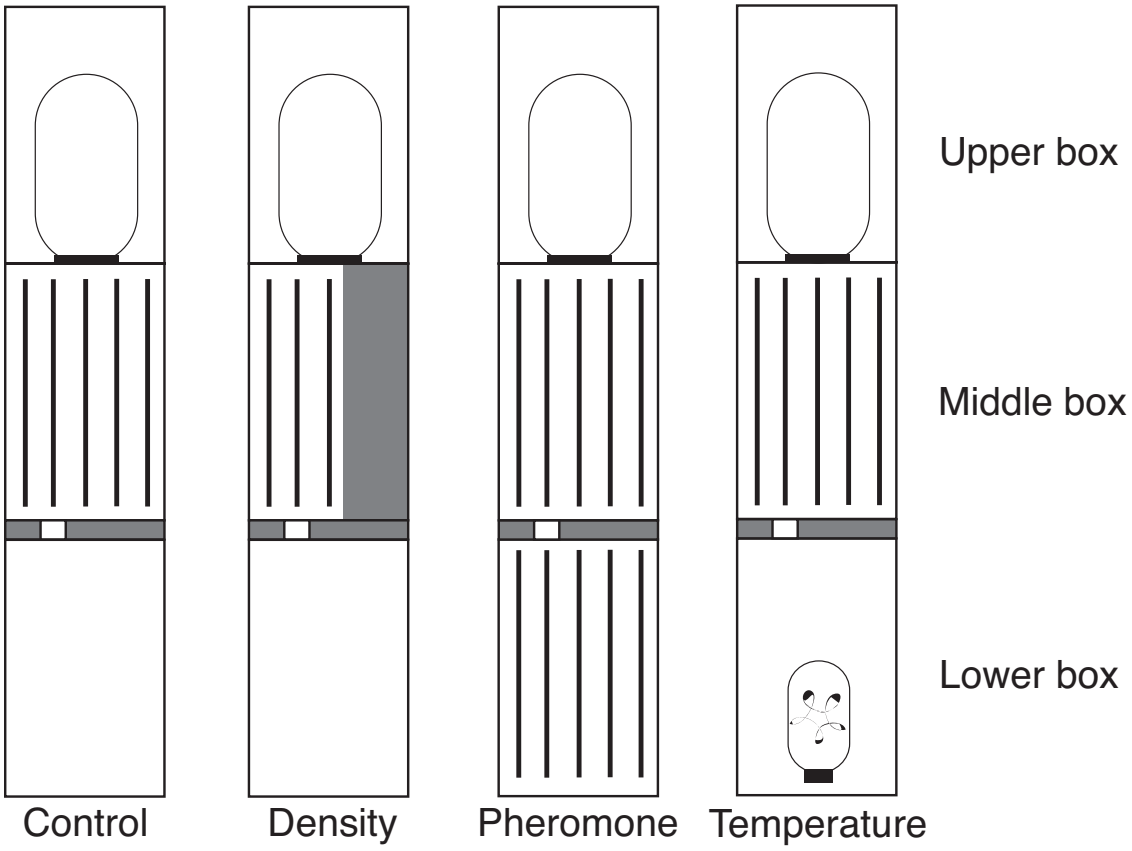

Figure 3.1 Experimental setup for manipulating potential cues of colony size. The middle box of each hive contained 8000 worker bees and a queen bee, 1 frame of brood, and 2 or 4 empty frames that provided space for comb building. Vertical lines in the boxes represent bee frames.

2. Colony Preparation and Installation

The control treatment group had 10 colonies and the other treatment groups had 8 colonies each. To equalize the number of worker bees per colony, each one was started as an artificial swarm made with worker bees taken from one of the Liddell Field Station apiaries, and a queen purchased in May 2014 from C.F. Koehnen and Sons (Ord Bend, CA, USA). Each artificial swarm had 1.07 kg ± 0.03 kg of worker bees —8200 ± 200 bees (Mitchell 1970; Otis 1982)—and was prepared following standard methodology (Seeley & Tautz 2001). The bees were fed 1:1 (vol:vol) sucrose solution ad libitum for 96 h before being installed in their hive.

On day 0, 22 June 2014, the 34 artificial swarms were randomly assigned to a treatment group. Each colony was given one frame of fully drawn worker comb whose cells were at least 60% filled with larvae and capped brood. This frame was placed in the middle of the box. The other frames given to each colony were empty, without comb or wax foundation, so they provided space for the workers to build whichever type of comb they wished. After being installed, the colonies were left undisturbed except on days 5 and 8 when we measured, using a 2 x 2 cm grid, the areas of worker comb and drone comb built in the initially empty frames. Drone comb is easily identified because the cells of drone comb are larger than those of worker comb (wall-to-wall distance: 6.2–6.9 mm vs. 5.2–5.7 mm) (Martin & Lindauer 1966; Taber & Owens 1970). We filled the sucrose feeder in each hives' upper box on days 0, 3, 5, and 8.

At the end of the experiment, 4 July 2014, we estimated the number of workers in each colony using the Liebefeld method (Imdorf et al. 1987). All colonies

decreased in size, to 5013 ± 812 bees, but none of the treatment groups were significantly different from the control (Tukey HSD, $P > 0.05$). This also confirmed that colonies in the pheromone treatment did not lose workers to the colony in the lower box.

3. Data Analysis

Statistical analyses were performed using R software version 3.1.1 and the packages lme4, lmerTest, and lsmeans (R Core Team 2014; Bates et al. 2015; Kuznetsova et al. 2014; Lenth 2016). Colonies with a dead or non-laying queen were removed from the analyses, so our final sample sizes were 10, 7, 7, and 8 colonies in the control, density, pheromone, and temperature treatments, respectively. The proportion of drone comb built was compared between treatments using a linear mixed-effects model, with colony ID as the random factor. The best-fit model was determined by comparing AIC values with a Chi-square test (Akaike 1974). The best-fit model included the interaction between treatment and experimental day (day 5 and day 8), and was significantly different from a null model that did not include these predictor variables ($P < 0.001$). We then performed pairwise comparisons with a Tukey adjustment to check for significant differences between the treatment groups.

Observational Study

The goal was to monitor and compare cues in small and large colonies to see which cues reliably change with colony size, and how. Presumably, cues more

tightly correlated with colony size are more likely to be used by workers to detect colony size. We set up four 4-frame observation hives: two with 5000 bees (small colony size: Colony SI and S2) and two with 10000 bees (large colony size: Colony L1 and L2). We monitored these colonies for 8 days.

1. Observation Hive Setup

The observation hives were built as shown in (Seeley 1995, p.73), but held four frames of comb instead of two. In order of top to bottom, the four frames contained: capped honey, capped brood, empty comb, and nothing (empty frame). All the cells in the capped honey frame were filled with honey. Most cells (80%) in the capped brood frame were filled with capped brood. All cells in the empty comb frame were empty. The empty frame had no comb at first; it provided a place for workers to build comb. None of the frames contained drone comb, because drone comb inhibits drone comb construction (Pratt 1998) and we wanted to confirm that the large colonies were large enough to build drone comb. On both sides of each observation hive, we drew on the glass a grid that divided each frame into eight equal regions. All 4 hives were oriented with their entrances facing north, and all were kept in the same climate-controlled building (room temperature ca. 25°C).

Four colonies from the Liddell Field Station apiary were selected as source colonies for stocking the observation hives. Each colony was headed by a queen purchased in May 2014 from C.F. Koehnen and Sons (Ord Bend, CA, USA). To have two cohorts of bees of known age in each observation hive colony, we removed from each source colony over 100 freshly emerged bees on 30 July and 7 August 2014, we

paint-marked these bees (Posca Paint Pens, Japan), and then we returned them to their respective colonies. On 11 August 2014, we collected from each source colony the following materials: the queen, one frame of brood, as many marked bees as possible (ca. 90-150 bees), and additional workers (5000 for small colonies, 10000 for large colonies, as determined by weight). After installing the queen, brood frame, and workers in each observation hive, we left the colonies undisturbed for one day, and then we began monitoring them on 13 August 2014. The cues that we monitored fell into three categories: worker density, worker behavior, and colony temperature.

2. Worker Density

A. Contact rate

To see if bees might use contact rate to assess colony size, we quantified contact rates between individuals in small and large colonies. From 13 August to 20 August 2014, we followed randomly chosen marked bees for 30-seconds, counting the number of times the focal bee contacted or was contacted by other bees. A contact was defined as any touching between the focal bee and another bee. We followed 20 individuals (10 from each age cohort) in each colony each day, for a total of 640 observations.

B. Antennation rate

To see if bees might use rate of antennations with other bees (i.e. antennae-to-antennae contacts, each lasting more than 1 s) to sense colony size, we assessed how the rate of antennations a bee experiences differs between small and large

colonies. From 13 August to 20 August 2014, we followed randomly chosen marked bees for 30-seconds, counting the number of times the focal bee made antennal contacts with other bees. We followed 20 workers (10 from each age cohort) in each colony each day, for a total of 640 observations.

C. Transect line

Workers move throughout their colony's nest. To see if the density of bees surrounding a moving worker differs between small and large colonies, we quantified worker density in multiple locations in our study colonies. On 14 August 2014, from 8am to 10pm, we photographed each observation hive every two hours. We then digitally drew two 20-cm horizontal transect lines across each frame (capped honey, capped brood, empty comb, empty frame), and counted the number of bees "touched" by this line. We pooled the number of bees along a transect line across all time points for each colony, after confirming that time of day did not significantly improve the statistical model (see below).

D. Worker velocity and turning angle

We digitally tracked individually marked bees to see if a worker bee's velocity changes with colony size. Using DLTdv5 software (Hedrick 2008), we digitized the paths of marked bees in videos taken on 13 August 2014. For each colony, we tracked as many marked bees as possible in a 1-minute video (Colony S1, 57 bees; Colony S2, 46 bees; Colony L1, 31 bees; Colony L2, 37 bees; total, 171 bees). To quantify the velocity of bees while moving (i.e., not while engaged in a task), we set a minimum threshold velocity of 0. 75 mm/s. This threshold was determined after assessing the digitized paths of a subset of non-moving bees.

A bee changes travel direction when her path is obstructed. To see if a bee moves differently depending on colony size, we quantified the turning angles for workers in small and large colonies. Using the digitized path data, we calculated the turning angle at each time point when a bee moved, relative to the previous and subsequent time points. This calculation does not distinguish between bees turning left versus turning right, but rather compresses turning angles between 0 and 180°.

3. Worker Behavior

A. Tasks

Workers perform tasks throughout the nest. To see how workers adjust their efforts among tasks as a function of colony size, we monitored the locations and the behaviors of marked bees from two age cohorts. From 13 August to 20 August 2014, we scan sampled both sides of each observation hive and recorded the location, task, and age cohort of each marked bee that we spotted. On the first day of data collection, the bees in the older age cohort were 15 days old and the bees in the younger age cohort were 7 days old. We identified tasks as in (Kolmes 1984), with some modifications (Table 3.S1).

To analyze these data, we sorted the 43 specific tasks into 7 general tasks: walking, resting, nursing, hive maintenance, worker maintenance, in festoon, and foraging. A festoon is a cluster of bees hanging attached to one another; it resembles a curtain and often surrounds an area where comb is under construction. We tested whether bees were engaged in each of the 7 general tasks differently based on colony size (large or small) and age cohort (old and young) using a binomial

generalized linear mixed effects model. We accounted for the age of the marked bees, because bees change tasks according to their age (Seeley 1982). Experimental day and colony ID were set as random factors. To further test whether bees in small or large colonies began foraging at a younger age, we made experimental day a predictor variable, which did improve the model ($P < 0.05$), but was not significantly different for marked bees in small versus large colonies ($P > 0.05$).

B. Location

To see whether bees use their distribution throughout the nest to assess colony size, we determined whether bees in the small and large colonies used differently the space within their hives. For example, bees in large colonies might spend more time at the periphery of the nest than do bees in small colonies. Using the location data that were collected for examining the workers' task distributions, we summed the number of marked bees in every grid square each day, from 13 August to 20 August 2014. We then tested whether the locations of marked bees were different for workers in small and large colonies, and whether bees were more likely to be observed at the periphery or the center of the nest. We defined the nest periphery as the grid squares that touch the edges of the observation hive (40 per observation hive) and the nest center as the inner grid squares (24 per observation hive). We then tested for differences in the spatial distributions (periphery or center) for the marked bees in small and large colonies.

4. Colony Temperature

To see whether temperature differs with colony size, we placed iButtons on the center of both sides of each of the 4 frames in each observation hive. The iButtons logged temperature every 30 min from 08:00 h on 13 August 2014 to 12:00 h on 20 August 2014.

5. Data Analysis

All statistical analyses were performed using R software version 3.1.1 and the packages lme4, lmerTest, pbkrTest, and lsmeans (Bates et al. 2015; Kuznetsova et al. 2014; Halekoh & Højsgaard 2014; Lenth 2016). For each cue that we monitored, we built a generalized linear mixed effects model to test for differences between small and large colonies, with colony ID as the random factor. We then added fixed effects, such as colony size, and tested whether each fixed effect significantly improved the model versus a null model using AIC comparison and a Chi-square test (Akaike 1974). We then used an F-test with a Kenward-Roger approximation to determine the significance of a given fixed effect. If there were multiple predictor variables, we performed pairwise comparisons with a Tukey adjustment. Values are reported as mean ± SD.

RESULTS

Experimental Study

Every colony built comb, but not every colony built drone comb. Table 3.1 shows for each treatment group the mean amount of comb built and the mean proportion of this comb that was drone comb. Figure 3.2 shows the proportion of drone comb built in each treatment. Only in the density treatment is the proportion of drone comb significantly higher than in the control treatment ($P = 0.047$).

Table 3.1 Comb building by treatment group (mean ± SD).

Treatment	# of colonies	Total comb built (cm^2)	Drone comb built (cm^2)	Proportion drone comb
Control	10	1822 ± 359	50 ± 83	0.03 ± 0.04
Density	7	1572 ± 116	190 ± 178	0.12 ± 0.11
Pheromone	7	1969 ± 107	37 ± 68	0.02 ± 0.03
Temperature	8	1702 ± 397	42 ± 86	0.02 ± 0.05

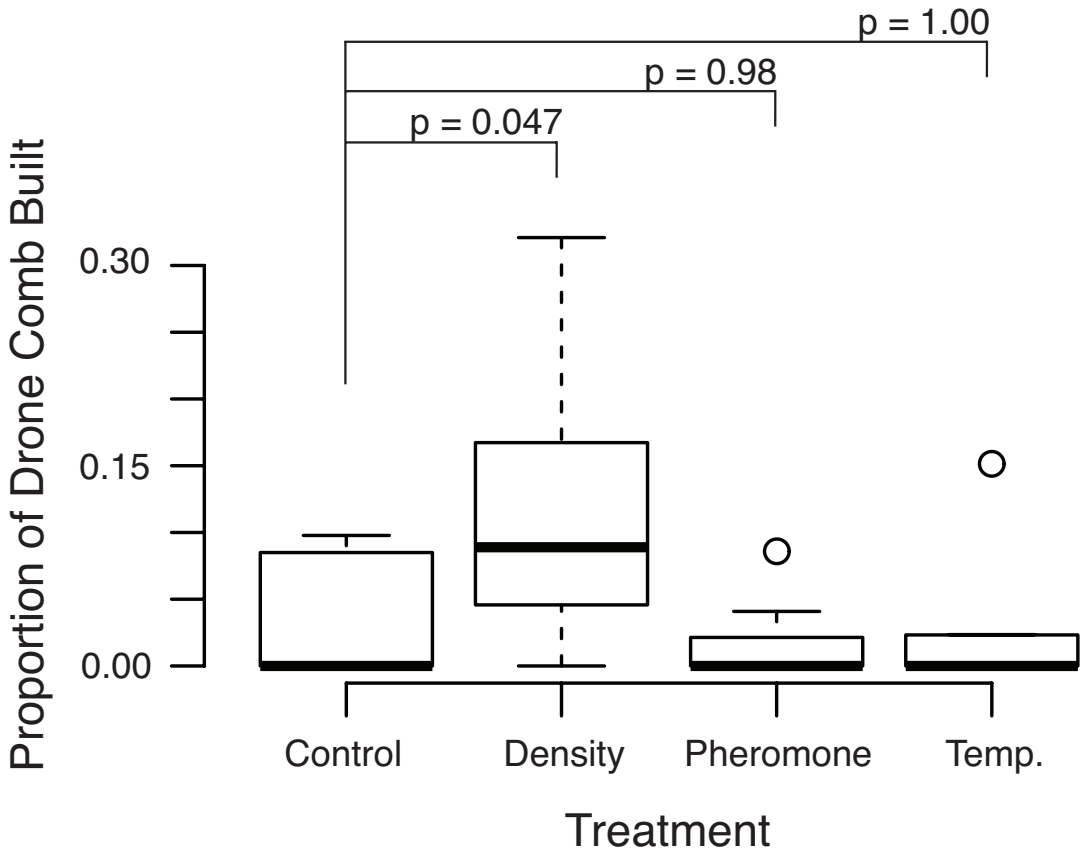

Figure 3.2 The proportion of drone comb built by the colonies in each treatment group.

Observational Study

Both large colonies built comb, including drone comb. Colony L1 built 24 cm^2 of worker comb and 594 cm^2 of drone comb; colony L2 built no worker comb and 10 cm^2 of drone comb. Neither small colony built any comb. Therefore, the two large colonies were above the threshold colony size needed to build drone comb, and the two small colonies were below the threshold colony size needed to build drone comb. The workers in the small colonies would likely have built worker comb had there not been empty comb available.

1. Worker Density

A. Contact rate

Bees in large colonies had significantly higher contact rates than bees in small colonies ($P = 0.016$). In small colonies, bees received 10.9 ± 6.6 contacts/30 s, whereas bees in large colonies bees received 14.9 ± 6.5 contacts/30 s. Adding the age cohort of the marked bees did not significantly improve the model over one that included only colony size ($P > 0.05$).

B. Antennation rate

Antennation rates between bees did not significantly differ between small and large colonies ($P > 0.05$). In small colonies, bees had 0.43 ± 0.76 antennations/30 s; in large colonies, they had 0.49 ± 0.78 antennations/30 s.

C. Transect line

When we compared the number of bees along 8 transect lines (Figure 3.3), we found that the location of the transect line was significant ($P < 0.001$), with the highest numbers of bees in the nest center, atop the capped brood and on empty comb frames. The interaction between transect-line location and colony size was also significant ($P < 0.001$). Large colonies had more bees along their transect lines than did small colonies ($P < 0.005$). Comparing the number of bees at *each* transect line between the small and large colonies (Figure 3.3), we found fewer bees in the small colonies than large colonies (pairwise comparisons, $P < 0.01$), except for lines 5 and 7, where there was no difference. When we examined how the number of bees along a transect line differed *within* small and large colonies, we found more variation in worker density in the nests of small colonies than large colonies (see letters in Figure 3.3). In large colonies, worker density was comparatively uniform throughout the nest; except for transect line 1, where there were fewer bees than elsewhere (Figure 3.3).

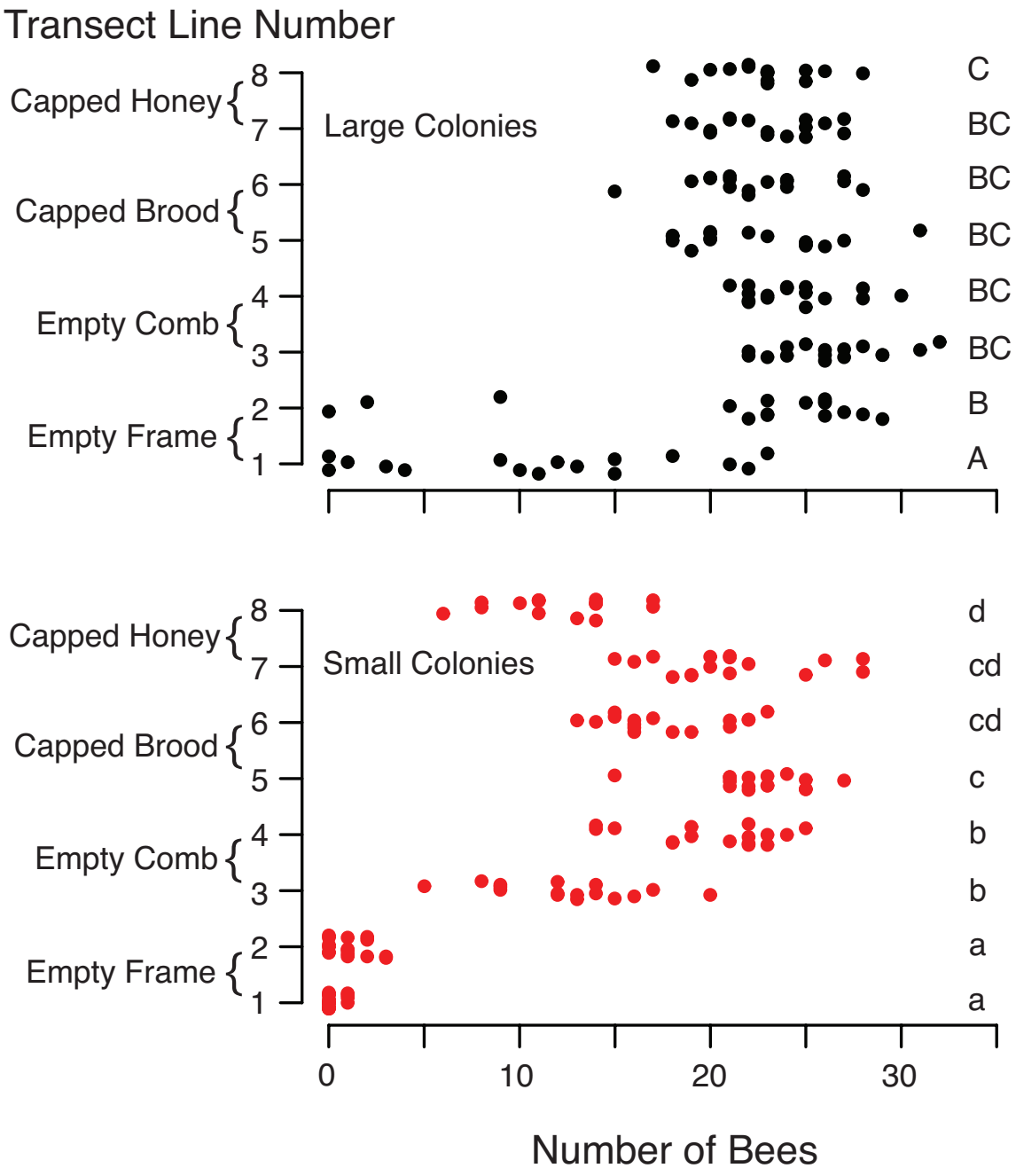

Figure 3.3 Numbers of bees observed along each transect line in large colonies (black points, upper plot) and small colonies (red points, lower plot). Transect lines are numbered from bottom of observation hive (1) to top (8). Letters along right axis denote statistical differences between the transect lines within large colonies or small colonies ($P < 0.05$). Along all transect lines, except 5 and 7, large colonies had more bees than did small colonies.

D. Worker velocity and turning angle

The mean velocities of bees in small and large colonies were not statistically different ($P > 0.05$). Bees in small colonies moved at 0.316 ± 0.149 cm/s; those in large colonies moved at 0.361 ± 0.140 cm/s. There was also no difference in the maximum velocities: bees in small colonies, 0.905 ± 0.510 cm/s; those in large colonies, 1.071 ± 0.438 cm/s ($P > 0.05$). Furthermore, the turning angles did not significantly differ between workers in small and large colonies ($P > 0.05$). In small colonies, bees moved at 77.3 ± 55.7°; in large colonies bees moved at 78.3 ± 56.9° (Figure 3.4).

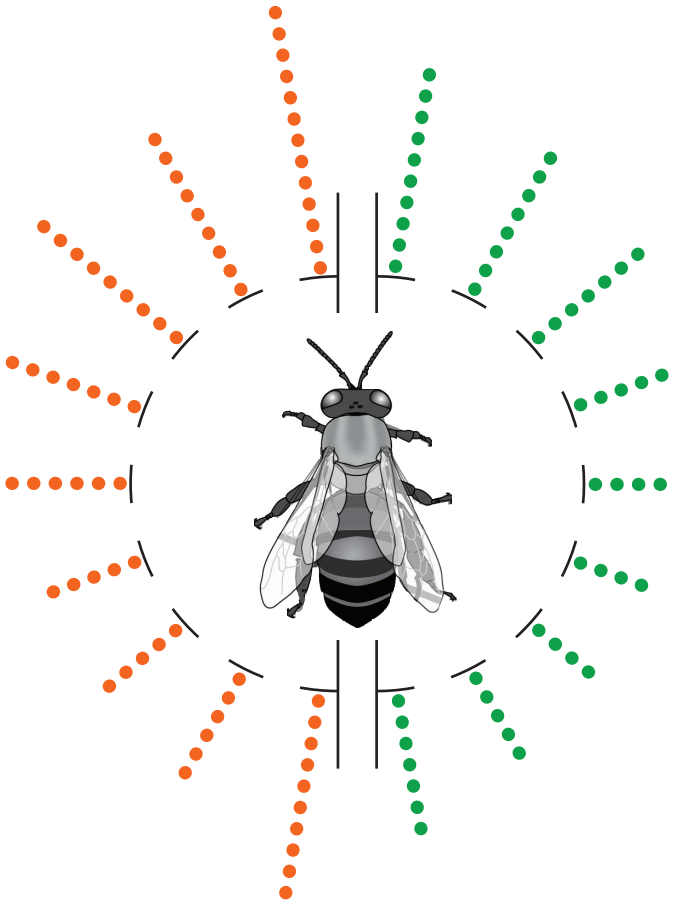

Figure 3.4 Worker turning angle for bees in small (left, orange dots) and large (right, green dots) colonies. Each dot represents 50 times that a marked bee turned by the angle shown. Calculating the turning angle did not distinguish between bees turning left and turning right, but rather compressed angles between 0 and 180°.

2. Worker Behavior

A. Tasks

We observed 3504 tasks being performed by the marked bees (Table 3.2). Walking, resting, hive maintenance, worker maintenance, and foraging behavior were performed with significantly different frequencies between the age cohorts ($P < 0.05$). The difference in frequency of nursing was marginally non-significant ($P = 0.056$). Frequency of being in a festoon was not significantly different between the age cohorts ($P > 0.05$). Comparing the tasks performed by bees in small and large colonies, only resting and being in the festoon were significantly different ($P < 0.05$). Bees in small colonies were observed resting more often than bees in large colonies. Bees in large colonies were observed in the festoon more often than bees in small colonies.

Table 3.2 The percent of time that marked bees were observed engaged in different tasks in relation to colony size and age cohort (counts in parenthesis).

Task	Small Colonies		Large Colonies		Significance	
	Old Cohort	Young Cohort	Old Cohort	Young Cohort	Colony Size?	Age Cohort?
Walking	30.9 (332)	24.1 (248)	31.4 (187)	27.6 (222)	$P = 0.68$	$*P = 0.0011$
Resting	12.1 (130)	15.5 (159)	5.9 (35)	7.8 (63)	$*P < 0.0001$	$*P = 0.037$
Nursing	2.6 (28)	3.8 (39)	3.5 (21)	5.7 (46)	$P = 0.12$	$P = 0.056$
Hive maintenance	35.5 (382)	45.6 (469)	39.4 (235)	40.9 (329)	$P = 0.97$	$*P = 0.0003$
Worker maintenance	14.7 (158)	9.1 (93)	10.9 (65)	11.7 (94)	$P = 0.99$	$*P = 0.019$
In festoon	0.9 (10)	0.9 (9)	7.4 (44)	5.1 (41)	$*P < 0.0001$	$P = 0.58$
Foraging	3.4 (36)	1.1 (11)	1.5 (9)	1.1 (9)	$P = 0.42$	$*P = 0.0054$
Total Counts	1076	1028	596	804		

B. Location

The marked bees were observed in 3504 locations. The locations of the marked bees did not significantly differ between small and large colonies ($P > 0.05$). When we categorized the bees' location as either at the periphery or the center of the observation hive, we still found no significant difference between small and large colonies ($P > 0.05$).

3. Colony Temperature

The overall nest temperature did not significantly differ between small and large colonies ($P > 0.05$) (Figure 3.5 and Table 3.3). Comparing the temperatures of each frame separately for the small and large colonies, we found that only for the lowest frame (empty frame) was there a significant difference ($P < 0.05$). In small colonies, the empty frame was 6.4°C cooler than in large colonies.

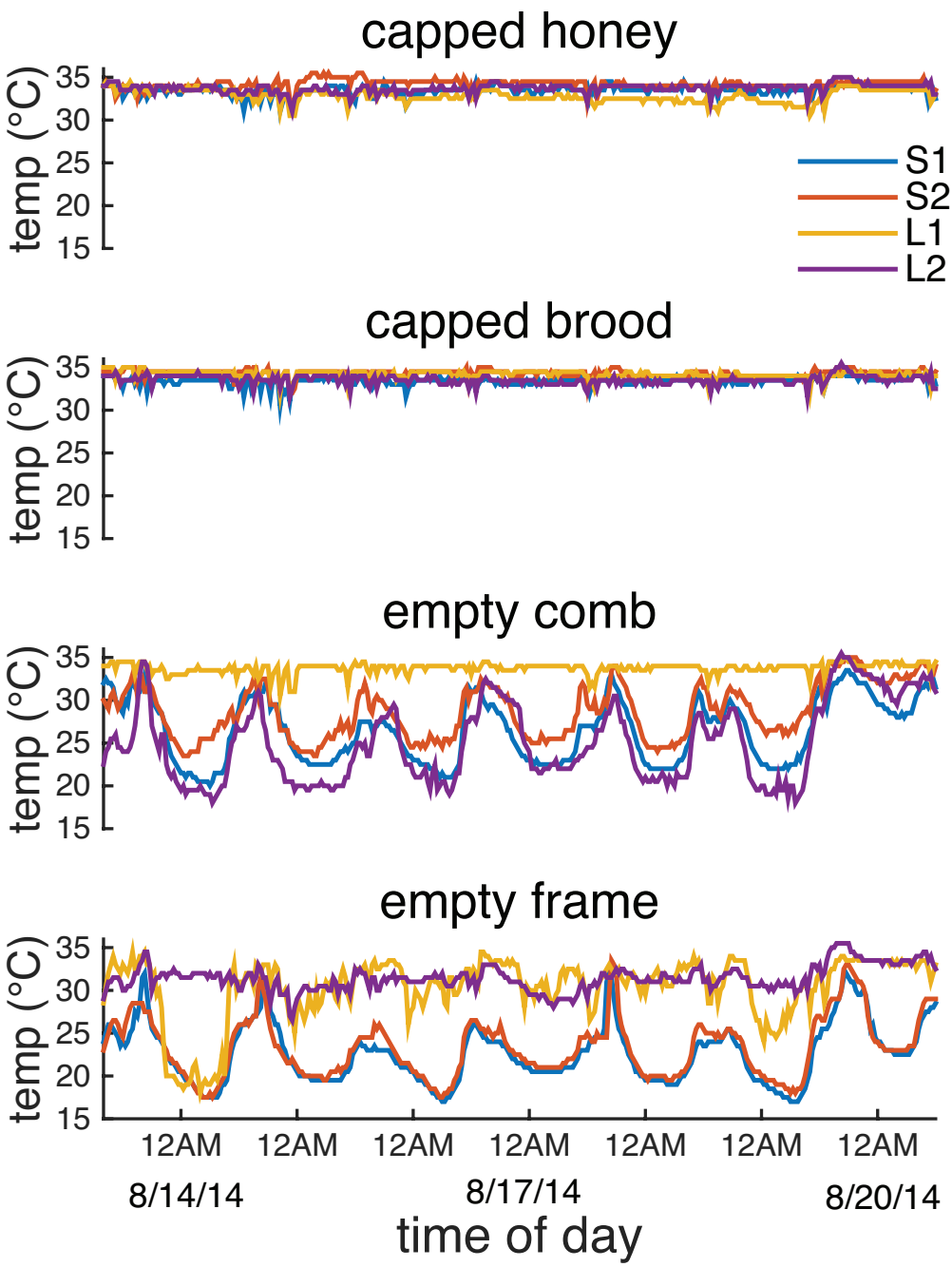

Figure 3.5 The temperature of the four frames in the observation hives. Each line denotes a single colony's data. The temperatures were significantly different between the small and large colonies only at the lower periphery of the nest, i.e. on the empty frame.

Table 3.3 Mean temperature measurements of frames in small and large colonies. Frames are ordered as in the observation hive, with capped honey at the top, and the initially empty frame (combless) at the bottom.

Frame	Temperature (°C) in small colonies	Temperature (°C) in large colonies	Significance
Capped honey	33.7 ± 0.7	33.1 ± 0.7	$P = 0.24$
Capped brood	34.0 ± 0.7	34.1 ± 0.6	$P = 0.87$
Empty comb	27.3 ± 3.7	31.0 ± 4.4	$P = 0.35$
Empty frame	22.9 ± 3.4	29.3 ± 4.3	$^{*}P = 0.023$
Mean nest temperature	29.5 ± 5.3	31.9 ± 3.6	$P = 0.15$

DISCUSSION

We used both experimental and observational approaches to investigate the cue(s) worker bees use to sense colony size. The experimental study increased three cues independently in an attempt to "trick" the bees into overestimating their colony's size, and thus above the reproductive threshold. The observational study monitored cues in small and large colonies to identify reliable indicators of increased colony size, hence candidates for the cue(s) that the bees use to sense their colony's size.

Table 3.4 Summary of results found in the experimental and the observational studies.

Experimental Study Cue Tested	Difference? (Y/N)	Notes
Increased worker density	Y	Increasing worker density increased the proportion of drone comb built.
Increased volatile pheromones	N	No effect.
Increased nest temperature	N	No effect.
Observational/Correlational Study		
Cue Examined	**Difference?**	**Notes**
Contact frequency	Y	Contact rate higher in larger colonies
Antennation frequency	N	No difference.
Worker density throughout nest	Y	Number of workers on transect lines is higher and less variable in larger colonies
Worker velocity	N	No effect of colony size on the mean velocity or max velocity of moving workers
Worker turning angle	N	No difference. Turning angles are nearly identical for individuals in small and large colonies.
Worker task distribution	Y/N	Workers in large colonies spend significantly less time resting, and more time in the festoon. All other behaviors were no different for workers in small and large colonies.
Worker location	N	No difference.
Colony temperature	Y/N	Higher temperature in large colonies, but only on the lowest frame.

Experimental study

Three potential cues of colony size were increased in the experimental study: density of workers, quantity of volatile pheromones, and temperature in the nest. Only an increase in worker density resulted in a higher proportion of drone comb built relative to the control colonies. These results suggest that workers somehow sense worker density and use this sensation to assess their colony's size. However, increased worker density may not be the critical cue per se; it seems likely that the critical cue varies *with* colony density and increases as worker density increases (e.g., contact rates, antennation frequencies, difficulty in movement). To explore which specific stimuli reliably change with colony size, and so might be used by the bees as cues of colony size, we conducted an observational study.

Observational study

We monitored potential cues in small and large colonies to determine which stimuli reliably change with colony size (Table 3.4). We grouped these potential cues into three categories: worker density, worker behavior, and colony temperature.

1. Worker Density

Experimentally increasing worker density was the only treatment that increased the proportion of drone comb built relative to the control (Figure 3.2). Therefore, for the observational study, we looked closely at stimuli that covary with worker density and that can be sensed by individual bees.

An individual bee experiences a higher frequency of contacts with other bees in a large colony than in a small colony. Given a set nest cavity size, a larger colony has more bees packed together, so we expect contact rates to increase, but workers in a colony with twice as many bees do not receive twice as many contacts (small colonies with 5000 bees: 10.9 ± 6.6 contacts per 30 s; large colonies with 10000 bees: 14.9 ± 6.5 contacts per 30 s). Also, bees do not distribute themselves uniformly in the nest, so contact rates will vary with worker density throughout the nest. At the colony level, we monitored worker density with the number of bees along 8 transect lines. We show that in the nest center the number of bees along a transect line is independent of colony size. At the nest periphery, however, there is a higher density of bees in larger colonies, suggesting that workers spread themselves uniformly over some comb surfaces, such as the brood nest in the center of the colony, and this pushes additional bees to the periphery. Therefore, if a worker were to walk from the nest center to periphery, she would experience greater variation in worker density (and presumably contact rate) in a small colony relative to a large colony. Bees patrol their nest widely (Johnson 2008), so variation in worker density could be used to assess colony size.

We found no difference in bee velocity as a function of colony size. Neither mean velocity nor maximum velocity of tracked bees differed between small and large colonies. This was surprising, given that larger colonies contain more individuals and so have more potential obstacles to a bee moving between two points. We therefore tested whether bees in large colonies are forced to turn more often than bees in small colonies, given that their velocity does not change. This also

turned out to be false: bees in small and large colonies have the same mean turning angles (Figure 3.4). It is unlikely, therefore, that workers use velocity or turning angle to sense colony size.

How likely is it that workers use contact rates to sense their colony's size? Simpson et al. (2001) showed that locusts sense the density of conspecifics in their immediate environment by the frequency of contacts they receive on the hind femur. Honey bee workers may do the same. Our results show that the density of workers throughout the nest changes more dramatically in a small colony than in a large colony, so if a worker receives a constant rate of contact as she walks through her colony, then she is likely in a large colony. If a worker receives a variable rate of contact as she moves through her colony, then she is likely in a small colony. This variation in contact rate might serve as a cue of colony size.

2. Worker Behavior

We tested whether worker tasks change with colony size. For example, do workers in small colonies spend more time nursing young larvae than workers in large colonies? If the tasks that workers are engaged in change with colony size, then workers might use this information to sense the size of their colony.

Of the 7 tasks we observed (condensed from 43 specific tasks into 7 general tasks: walking, resting, worker maintenance, hive maintenance, nursing, foraging, in festoon), only in festoon and resting were significantly different between small and large colonies. Workers in large colonies were found in the festoon significantly more often than workers in small colonies. This is because large colonies have more

bees with which to make a festoon than do small colonies. In the observation hives, the large colonies had large festoons, and the small colonies had small festoons or none at all. Could workers use the size of their festoon to determine whether they should begin building drone comb? Probably not. When a colony first inhabits a nest cavity, there are no combs yet, and so all the bees cluster together in a large festoon. Despite the large festoon, the workers first build worker comb, not drone comb (Smith et al. 2016). Workers presumably need to assess their colony's size in other situations as well, so we do not expect the size of a festoon to be a general indicator of colony size.

Workers in large colonies, relative to those in small colonies, also spend significantly less time resting, perhaps due to the higher contact rates in large colonies. Time spent inactive could be a way for a worker to sense her colony's size: the more time spent active, the larger the colony. While the mechanism is plausible, the trend seems unexpected. Michener's paradox states that per capita productivity decreases as colony size increases (Michener 1964), and so predicts that workers in large colonies spend more time inactive than workers in small colonies, the opposite of what we found (Table 3.2). Michener's study, however, measured the productivity of a specific task (number of capped brood cells). Other studies show increased productivity with colony size, and thus workers spend less time inactive, such as during nest building, in contrast to Michener's Paradox (e.g. Jeanne 1986; Jeanne & Nordheim 1996; see also Karsai & Wenzel 1998; Strohm & Bordon-Hauser 2003; Bouwma et al. 2006). To the best of our knowledge, our study is the first to look broadly at worker's task performance in relation to colony size, so it is the first

to show that workers in large colonies spend less time resting, at least in *Apis mellifera*. Our study, however, only compares two small colonies with two large ones, and our definition of inactive bees (resting) includes all bees that were immobile. It is possible that some of these bees appeared inactive, but actually were heating the colony by contracting their flight muscles (Esch 1960). Even so, by our definition, there were twice as many bees resting in small colonies than in large colonies, so workers could use the amount of time spent resting as a measure of colony size: the less time you rest, the larger your colony.

The worker task data also gave us information about the marked bees' locations in the nest. We tested whether workers in small and large colonies used the space within their nests differently, but found no differences. This indicates that although worker density varies in the nest, individuals are still moving throughout the entire nest.

3. Colony Temperature

We monitored the temperature of colonies to quantify temperature variation with colony size. This was done to determine if workers in small and large colonies experience different temperature gradients while moving through their nests. We found no difference in temperature at the center of the observation hive in the small and large colonies, which corroborates existing studies that show honey bees tightly regulate the temperature of the brood nest (Seeley 1985). However, small colonies were significantly cooler than large colonies on the lowest periphery of the observation hive. This is because large colonies have more bees than do small

colonies, and each bee has a resting metabolism that keeps her a few degrees above ambient temperature (Kovac et al. 2007). Therefore, we expect large colonies to be warmer than small colonies simply due to a larger number of heat-emitting individuals. The experimental study, however, showed that increased temperature does not induce workers to build drone comb, so we do not expect bees to use nest temperature to sense colony size.

Conclusions

The experimental study found that worker bees responded to an increase in worker density by producing more drone comb in preparation for reproduction. The observational study measured potential cues of colony size in small and large colonies living in nests of the same size, hence with relatively low and high densities. We found that worker contact rate increases with colony size, and that worker density is less variable in large colonies. Workers in large colonies also spend less time resting than workers in small colonies, which may contribute to the increase in contact rate. Therefore, the observational study found contact rate to be the most likely indicator of colony density for honey bees.

The ability to sense developmental state is critically important for all organisms, including organisms whose development depends on group size. For a honey bee colony, workers evidently use density to sense group size. The underlying cue of worker density remains uncertain, but presumably it is a physical cue related to worker movement, although we did not test for non-volatile chemical cues. Whereas groups of unicellular bacteria and multicellular animals use chemical

cues to sense their size, the one superorganism where its been studied, a honey bee colony, evidently relies on physical cues to sense group size.

KEYWORDS

Development, sociogenesis, reproductive investment, honey bee, *Apis mellifera*, colony size

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Table 3.S1 Honey bee activity codes, based on Kolmes 1984, with additional tasks added and their order reorganized. The 7 general task descriptions were for statistical analyses only.

Code	Specific Task	General Task
1	Walking	Walking
2	Resting	Resting
3	Grooming self	Worker maintenance
4	Grooming other	Worker maintenance
5	Groomed by other	Worker maintenance
6	Inspecting empty/egg cell	Hive maintenance
7	Inspecting larvae	Nursing
8	Into pollen cell	Hive maintenance
9	Into honey cell	Hive maintenance
10	Cleaning cell (deep in cell, abdomen moving, bee is rotating)	Hive maintenance
11	Sleeping (deep in cell, not rotating, abdomen is pulsating)	Resting
12	Feeding worker (other bee's proboscis between focal bee's mandibles)	Worker maintenance
13	Fed by worker (proboscis extended)	Worker maintenance
14	Beg for food (worker antennates another, exchange food)	Worker maintenance
15	Antennate with worker, no food exchanged	Worker maintenance
16	Attend queen	Worker maintenance
17	Feed queen	Worker maintenance
18	In festoon	In festoon
19	Building comb with new wax	Hive maintenance
20	Mouthing sealed brood	Nursing
21	Mouthing sealed honey	Hive maintenance

22	Chew on wood in hive	Hive maintenance
23	Chew on wax in hive	Hive maintenance
24	Working with propolis	Hive maintenance
25	Chew pollen on worker	Hive maintenance
26	Uncap brood	Worker maintenance
27	Capping brood	Nursing
28	Capping honey	Hive maintenance
29	Extend mouthparts to ripen honey (fluid bubble at end of proboscis)	Hive maintenance
30	Fanning	Hive maintenance
31	Undertaker (holding a dead bee)	Hive maintenance
32	Tremble dance (lateral wiggle resembling the dance of St. Vitus)	Foraging
33	Shaking signal (rapid up and down atop another bee)	Foraging
34	Waggle dance (forager)	Foraging
35	Attending waggle dance	Foraging
36	Guarding at entrance	Foraging
37	Orientation flight (zigzag at entrance, but bee does not depart)	Foraging
38	Exiting hive	Foraging
39	Entering hive	Foraging
40	Returning forager with pollen	Foraging
41	Returning forager with propolis	Foraging
42	Unloading nectar	Foraging
43	Foraging outside of the colony	Foraging

REFERENCES

Akaike H (1974) A new look at the statistical model identification. IEEE T Automat Contr 19(6):716–723. doi:10.1109/TAC.1974.1100705

Bates D, Maechler M, Bolker B, & Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67(1):1–48. doi:10.18637/jss.v067.i01

Boes KE (2010) Honeybee colony drone production and maintenance in accordance with environmental factors: an interplay of queen and worker decisions. Insect Soc 57(1):1–9. doi:10.1007/s00040-009-0046-9

Bouwma AM, Nordheim EV, Jeanne, RL (2006) Per-capita productivity in a social wasp: No evidence for a negative effect of colony size. Insect Soc 53(4):412–419. doi:10.1007/s00040-005-0886-5

Esch H (1960) Über die Körpertemperaturen und den Wärmehaushalt von *Apis mellifica*. Z Vergl Physiol 43:305–335. doi: 10.1007/BF00298066

Grumbach MM (2002) The neuroendocrinology of human puberty revisited. Horm Res 57:2–14. doi:10.1159/000058094

Halekoh U, & Højsgaard S (2014) A Kenward-Roger approximation and parametric bootstrap methods for tests in linear mixed models - the R package pbkrtest. J Stat Softw 59(9):1–30

Hedrick TL (2008) Software techniques for two- and three-dimensional kinematic measurements of biological and biomimetic systems. Bioinspir Biomim 3(3):034001. doi:10.1088/1748-3182/3/3/034001

Hölldobler B, Wilson EO (2009) The superorganism: the beauty, elegance, and strangeness of insect societies. New York, NY: WW Norton & Company

Imdorf A, Buehlmann G, Gerig L, Kilchenmann V, Willie H (1987) Überprüfung der Schätzmethode zur Ermittlung der Brutfläche und der Anzahl Arbeiterinnen in freifliegenden Bienenvölkern. Apidologie 18(2):137–146

Jeanne RL (1986) The organization of work in *Polybia occidentalis*: costs and benefits of specialization in a social wasp. Behav Ecol Sociobiol 19(5):333–341. doi:10.1007/BF00295706

Jeanne RL, Nordheim EV (1996) Productivity in a social wasp: per capita output increases with swarm size. Behav. Ecol. 7(1):43–48. doi:10.1093/beheco/7.1.43

Johnson BR (2008) Global information sampling in the honey bee.

Naturwissenschaften 95:523–530. doi:10.1007/s00114-008-0354-3

Karsai I, Wenzel JW (1998) Productivity, individual-level and colony-level flexibility, and organization of work as consequences of colony size. Proc Natl Acad Sci USA 95(15):8665–8669

Kolmes SA (1984) A quantitative comparison of observational methodologies for studies of worker honeybees. J Apicult Res 23(4):189–198. doi:10.1080/00218839.1984.11100631

Kovac H, Stabentheiner A, Hetz SK, Petz M, Crailsheim K (2007) Respiration of resting honeybees. J Insect Physiol 53(12):1250–1261. doi:10.1016/j.jinsphys.2007.06.019

Kuznetsova A, Brockhoff PB, Christensen R (2014) lmerTest: Tests in linear mixed effects models. R Package 2.0-20

Lenth RV (2016) Least-squares means: the R package lsmeans. J Stat Softw 69(1):1–33. doi:10.18637/jss.v069.i01.

Martin H, Lindauer M (1966) Sinnesphysiologische Leistungen beim Wabenbau der Honigbiene. Z Vergl Physiol 53:372–404.

Michener CD (1964) Reproductive efficiency in relation to colony size in hymenopterous societies. Insect Soc 11(4):317–342.

Mitchell C (1970) Weights of workers and drones. Am Bee J 110:468–469

Otis GW (1982) Weights of worker honeybees in swarms. J Apicult Res 21(2):88–92. doi:10.1080/00218839.1982.11100520.

Pratt SC (1998) Decentralized control of drone comb construction in honey bee colonies. Behav Ecol Sociobiol 42:193–205.doi:10.1007/s002650050431

R Core Team (2014) R: A language and environment for statistical computing. Vienna, Austria.

Seeley TD (1982) Adaptive significance of the age polyethism schedule in honeybee colonies. Behav Ecol Sociobiol 11(4):287–293. doi:10.1007/BF00299306.

Seeley TD (1985) Honeybee ecology: a study of adaptation in social life. Princeton NJ: Princeton University Press

Seeley TD (1995) The Wisdom of the Hive. Cambridge MA: Harvard University Press.

Seeley TD, Morse RA (1976) The nest of the honey bee (*Apis mellifera* L.). Insect Soc

23(4):495–512

Seeley TD, Tautz J (2001) Worker piping in honey bee swarms and its role in preparing for liftoff. J Comp Physiol A 187(8):667–676. doi:10.1007/s00359-001-0243-0.

Simpson SJ, Despland E, Hägele BF, Dodgson T (2001) Gregarious behavior in desert locusts is evoked by touching their back legs. P Natl Acad Sci USA 98(7):3895–7. doi:10.1073/pnas.071527998.

Slessor KN, Winston ML, Le Conte Y (2005) Pheromone communication in the honeybee (*Apis mellifera* L.). J Chem Ecol 31(11):2731–2745. doi:10.1007/s10886-005-7623-9.

Smith ML, Ostwald MM, Loftus JC, Seeley TD (2014) A critical number of workers in a honeybee colony triggers investment in reproduction. Naturwissenschaften 101(10):783–790. doi:10.1007/s00114-014-1215-x

Smith ML, Ostwald MM, Seeley TD (2016) Honey bee sociometry: tracking honey bee colonies and their nest contents from colony founding until death. Insect Soc doi:10.1007/s00040-016-0499-6

Strohm E, Bordon-Hauser A (2003) Advantages and disadvantages of large colony size in a halictid bee: the queen's perspective. Behav Ecol 14(4):546–553. doi:10.1093/beheco/arg039

Taber S, Owens CD (1970) Colony founding and initial nest design of honey bees, *Apis mellifera* L. Anim Behav 18:625–632

Waters CM, Bassler BL (2005) Quorum sensing: cell-to-cell communication in bacteria. Annu Rev Cell Dev Bi 21:319–46

CHAPTER 4

LARGER BUT NOT LOUDER: BIGGER HONEY BEE COLONIES HAVE QUIETER COMBS

Michael L Smith, Po-Cheng Chen

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ABSTRACT

Communication is impossible if the sender's signal cannot overcome background noise to reach the receiver. This obstacle is present in all communication modalities, forcing organisms to develop diverse mechanisms to overcome noise. Honey bees will modify combs to improve signal efficiency of substrate-borne vibrations, but it is unknown whether, and if so, how, bees compensate for the largest potential source of noise: the bees themselves. The number of bees in a colony changes markedly throughout the year, but the size of the nest cavity does not, forcing workers into high densities on the combs. How, then, do bees communicate via substrate-borne vibrations on combs that are covered in bees? We used accelerometers to measure comb vibrations, while varying the number of workers on the comb. Surprisingly, comb vibrations decreased with increased worker number. Furthermore, inserting freshly killed bees to the comb demonstrated that it is not simply the bees' collective mass that damps vibrations, but is probably their behavior. We propose that their posture damps vibrations, with each bee linking up to six neighboring cells with her legs. This collective damping reduces background noise, and improves the landscape for communication. These results demonstrate

how living systems, including superorganisms, can overcome physical obstacles with curiously simple and elegant solutions.

SIGNIFICANCE STATEMENT

Background noise is a pervasive problem in communication. Honey bees must address this problem be- cause thousands of individuals occupy and communicate within a single nest made of beeswax combs. While it is known that bees use beeswax comb vibrations to communicate, it is unknown how they overcome background noise when the combs become covered in bees. We show that comb vibrations decrease, not increase, as the number of bees on the comb increases. This unexpected result is not due to bees' mass, but rather their interactions with the comb that damps vibrations. By reducing background vibrations, workers make the comb quieter and improve the substrate for communication. Therefore, we show that the communication landscape for sending signals within the superorganism is improved, not hindered, as the colony grows.

INTRODUCTION

A flashy bird singing its song is both eye-and-ear-catching, but insects producing subtle signals via substrate-borne vibrations are also fascinating (reviewed by Cocroft and Rodríguez 2005; in social insects Hunt and Richard 2013). From anti-predator defense (Cocroft 1996), to geographically specific courtship (Miklas et al. 2003), to biased worker development (Suryanarayanan et al. 2011), substrate-borne vibrations are pervasive in insect communication. No matter the modality,

however, communication is only possible if the sender's signal can overcome background noise to reach the receiver.

The honey bee, *Apis mellifera*, also uses vibrations as part of its communication repertoire, for example, to advertise food sites with waggle dances (Nieh and Tautz 2000), to warn of danger with stop signals (Nieh 2010), and to mediate queen-worker interactions (Schneider et al. 2001; Pierce et al. 2007). While some vibrations are sent directly from one bee to another, many are transmitted to a larger audience through the wax combs (reviewed in Kirchner 1993). Sending signals through the comb adds unique challenges, because the nest changes as the colony grows and develops (Smith et al. 2016). Empty cells, for example, transmit vibrations better than sealed brood cells (Sandeman et al. 1996), so when the contents of a comb change, so too do the vibrations sent through the comb (Bencsik et al. 2015).

Bees modify combs to maximize signal efficiency. The waggle dance, used to recruit foragers, is performed on a dance floor on the comb near the hive's entrance (von Frisch 1967). Bees tend to keep this area free of brood, because foragers dancing on open cells attract recruits more effectively, and from farther away (Tautz 1996; Tautz and Rohrseitz 1998). Workers will even chew away edges of comb to free it from supporting structures and increase the efficacy of substrate-borne vibrations (Sandeman et al. 1996).

While bees can adjust their combs to improve signal transmission, this does not deal with what might be the largest source of signal interference – the bees themselves. Vibrational background noise could affect the efficacy of not only the waggle dance, but all forms of communication that rely on comb vibrations (Kirchner 1993). Is vibrational "noise" caused by thousands of bees moving and interacting on the comb a problem, and if so, how do the bees overcome this obstacle?

The number of bees in a colony is extremely variable, for it can range from only 3000 workers in early spring, to over 20000 in the summer (Smith et al. 2016). Whereas bees can build additional combs as their colony grows, they cannot enlarge their nest cavity, so eventually they can become more crowded. Indeed, as the number of bees in a colony increases, their density also increases (Smith et al. 2017). Given that bees modify their combs to improve the transmission of substrate-borne vibrations, perhaps they also have mechanisms to offset changes in comb vibrations caused by increased density of bees on the comb. We hypothesized that comb vibrations change with the number of bees on the comb, and that bees have mechanisms to offset this change so that their combs can be used for communication even when covered in bees.

This hypothesis was tested experimentally by varying the number of bees on a natural beeswax comb and measuring the comb vibrations with an accelerometer. We then determined how the number of bees on a comb alters the background noise in comb vibrations. When we found that an increase in the number of bees actually

caused a reduction in comb vibrations, we tested whether this reduction was due to an increase in the mass of the bees on the comb. We found that the sheer mass of the bees on a comb did not influence comb vibrations, which suggests that the worker's behavior damps comb vibrations.

METHODS

For each colony (n = 5), we measured comb vibrations as a function of the numbers of bees on the comb. First, we varied the number of bees on the comb by manipulating the number of bees that we added to a colony living in an observation hive. Second, we measured comb vibrations throughout the day, as the number of bees on the comb varied naturally.

Observation hive setup

Each observation hive (internal dimensions: 46 x 96.5 x 4.5 cm) held four wooden frames containing a beeswax comb (43 x 20 cm). The top three frames were standard Langstroth frames with wax foundation and stabilizing wires. The lowest frame (hereafter "the focal comb") contained an area of comb that was built naturally by the bees before the experiment (no wires, wax foundation, etc.), and so was more representative of combs found in natural nests. Most importantly, the focal comb had no wires to artificially stabilize the comb in the wooden frame, because unnatural materials can affect comb vibrations (Seeley et al. 2005). To measure comb vibrations on the focal comb, we pressed the "green board" (3.2 x 5.5 cm) holding the accelerometer into the comb, with a protective wire cage around

the accelerometer (5 x 6 cm). The wire cage kept the bees from the accelerometer on the first day, but the bees were able to chew around it and could contact the accelerometer within a day or two. The observation hives were kept in a climate-controlled room (20 °C), but the bees had free access to forage in the surrounding countryside.

Installing bees and data collection

To obtain bees for installation in the observation hive, we went to a source colony, put its queen into a cage (8 x 3 x 2 cm), and then shook equal weights of workers into two screened boxes (40 x 15 x 22 cm) to bring back to the observation hive at the Liddell Field Station of Cornell University in Ithaca NY (42°27.6'N, 76°26.7'W). The sizes of the worker groups were allowed to vary, but the two boxes of workers from one hive were always made equivalent to create a paired design, while still allowing for the variation in colony size that was used in the experiment. The number of workers in each box was determined by weighing a subset of 30+ workers. Before installing the bees in the observation hive, we took two baseline measurements of comb vibrations (to check for potential variation). We then released the queen and the workers from one of the two boxes into the observation hive (i.e. half the colony's workers = small-colony treatment). After giving the bees 1 hr to adjust, we measured the comb vibrations, and the number of bees on the focal comb (see below). Next, we added the workers from the second box, to create a large-colony treatment, allowed the bees to settle for 1 hr, and again measured comb vibrations and the number of bees on the focal comb.

After 24-48 hrs we measured comb vibrations throughout the day. For each colony, we measured comb vibrations at least five times throughout the day (06:00 to 24:00 h), and assessed the number of bees on the focal comb (see below). We also took additional comb vibration measurements, but without measuring the number of workers on the focal comb. This allowed us to determine whether comb vibrations change with time of day. We repeated this method for 5 colonies total (colonies installed on 5 July, 21 July, 28 July of 2015; 8 July and 28 August of 2016). The colony installed on 28 August 2016 was only used for measuring vibrations throughout the day.

Measuring the number of bees on the focal comb

We used a 4 x 4 cm grid to estimate the number of bees on either side of the focal comb (as in Smith et al. 2016). We only counted bees that were on the comb, whether walking, standing still, or inside a cell. We took 45 measurements of the number of bees inside the grid, calculated an average, and then multiplied by the comb area to determine the number of bees on the focal comb.

Measuring comb vibrations with the accelerometer

We used a smart, low-power, three-axis capacitive MEMS accelerometer to measure comb vibrations up to a bandwidth of 400 Hz (Product No: MMA8452Q, Freescale Semiconductor, Austin TX, now NXP Semiconductors). The accelerometer was connected to a I2C interface with a microcontroller (Product No: ATmega32U4,

Atmel, San José, CA). The raw output data were collected with MATLAB (R2015b, version 8.6), using a serial interface from the microcontroller. Comb vibrations were measured over 2 minutes, during which the accelerometer collected a total of 12000 data points (100 data points collected in $1/8^{th}$ of a second, once per second). To minimize observer bias, blinded methods were used when comb vibrations were recorded and analyzed.

Whole-day baseline

To ensure that our baseline measurements were not influenced by the time of day, we measured the comb vibrations in the observation hive setup, without bees, nine times, spaced between 07:00 and 22:00 on 1 August 2015. The whole-day baseline was performed in the same observation hive setup that was used for all experimental tests.

Dead bees on the comb trial

To determine whether the mass of the bees influenced comb vibrations, we placed freshly killed bees into the cells of a focal comb, and measured comb vibrations. On 14 December 2015, an uncharacteristically warm day, we captured bees at the entrances of source colonies and freeze killed them. After taking a baseline measurement, we inserted the fresh killed bees head first into cells of the focal comb, which had no live bees on it. The dead bees were distributed evenly on both sides of the focal comb. We took measurements of comb vibrations with 400, 600, 800, 1000, 1200, 1400, and 1600 dead bees. We then took nine additional

measurements of comb vibrations as we removed batches of dead bees until no more dead bees were on the focal comb (160 ± 50 dead bees per removal).

Visualizing and analyzing comb vibrations

To visualize the comb vibrations, we first calculated the magnitude of the 3-axis acceleration ($sqrt(x^2+y^2+z^2)$), and then made Power Spectrum Density plots of magnitude against frequency. Plots were made using the Welch PSD command in the R package bspec (Roever 2015). We then visually compared these plots to determine whether certain frequencies increased in power with increased numbers of bees on the focal comb, relative to the baseline measurements with 0 bees.

We also used unbiased methods to compare comb vibrations. We first converted the accelerometer .txt file to a .wav file using MATLAB, and then analyzed the .wav file in Raven Pro 1.4 (www.birds.cornell.edu/raven). We measured the following acoustic parameters for each recording: aggregate entropy, average entropy, average power, max power, energy, center frequency, max frequency, max amplitude, and root mean squared amplitude (Charif et al. 2010). We then plotted these values against the number of bees on the focal comb, and performed statistical analyses to determine if there was a relationship between the acoustic measurements and the number of bees on the focal comb.

For the statistical analysis, we made generalized linear mixed effects models, using the LME4 package in R (R Core Team 2014; Bates et al. 2015). We tested each

acoustic measurement (response variable, e.g. aggregate entropy) against the number of bees on the focal comb (predictor variable). Colony number was the random factor. We first determined whether adding the number of bees on the focal comb significantly improved the model versus a null model that did not include the number of bees on the focal comb. The best-fit model was determined by using likelihood ratio tests (Lewis et al. 2011) implemented in R using the anova() function. If the model was significantly improved, we then used a Type II Wald Chi Square Test to determine whether the number of bees on the focal comb significantly predicted the response variable. This same analysis was done to determine whether the time of day changed comb vibrations.

RESULTS

Power Spectrum Density Analysis

There was no clear association between number of bees on the focal comb and Power Spectrum Density (PSD) plots (see Figure 4.1). Having more bees on the focal comb did not change the frequencies at which the comb vibrated. Even when over 1000 bees were on the comb, the PSD plots were generally no different from the baseline, with 0 bees on the comb (Figure 4.1A). In some trials, it appeared that certain frequencies might increase in power with more bees on the comb (Figure 4.1B), but these patterns were not consistent (Figure 4.1C), even within the same trial. Therefore, the number of bees on a comb did not reliably change the frequency of the comb vibrations.

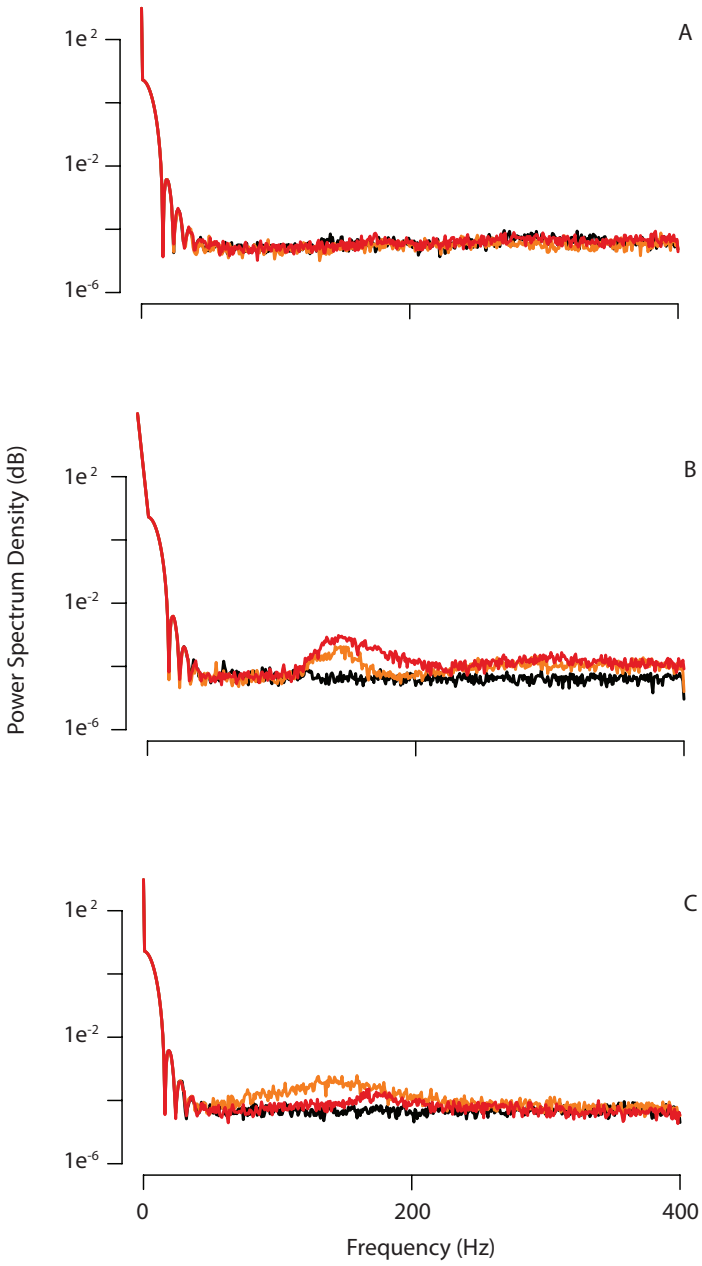

Figure 4.1 Examples of power spectrum density plots, demonstrating no clear pattern between the number of bees on the comb, and the frequency at which combs vibrate. Some trials show that the number of bees on the focal comb did not alter the vibration frequencies (A: black line, 0 bees on comb; orange line, 478 bees on comb; red line, 1150 bees on comb). Other trials suggested that having more bees increased the power of certain frequencies, for example, 150-250 Hz vibrations increased with the number of workers on the comb (B: black line, 0 bees on comb; orange line, 850 bees on comb; red line, 1130 bees on comb). Other examples show that comb vibrations do vary, but the pattern is not consistent with increasing worker number (C: black line, 0 bees on comb; orange line, 880 bees on comb; red line, 1150 bees on comb).

Acoustic Analysis

To compare comb vibrations using an unbiased method, we tested whether the number of bees on the focal comb changed the acoustic parameters of the comb vibrations. The number of bees on the focal comb did not significantly change the following acoustic parameters: aggregate entropy, average entropy, energy, center frequency, max frequency, or max amplitude ($P > 0.05$). In figure 4.2, however, we see that average power, maximum power, and root mean squared amplitude all significantly decreased as the number of bees on the focal comb increased (Generalized linear mixed-effects model: $P < 0.0001$, Pearson's product-moment correlations: average power, $r(61) = -6.04$, $P < 0.0001$; maximum power, $r(61) = -5.96$, $P < 0.0001$; root mean squared amplitude, $r(61) = -6.36$, $P < 0.0001$). Therefore, the number of bees on the comb did change the acoustic parameters of the comb vibrations, but, curiously, the parameters *decreased* as the number of bees moving and interacting on the comb increased.

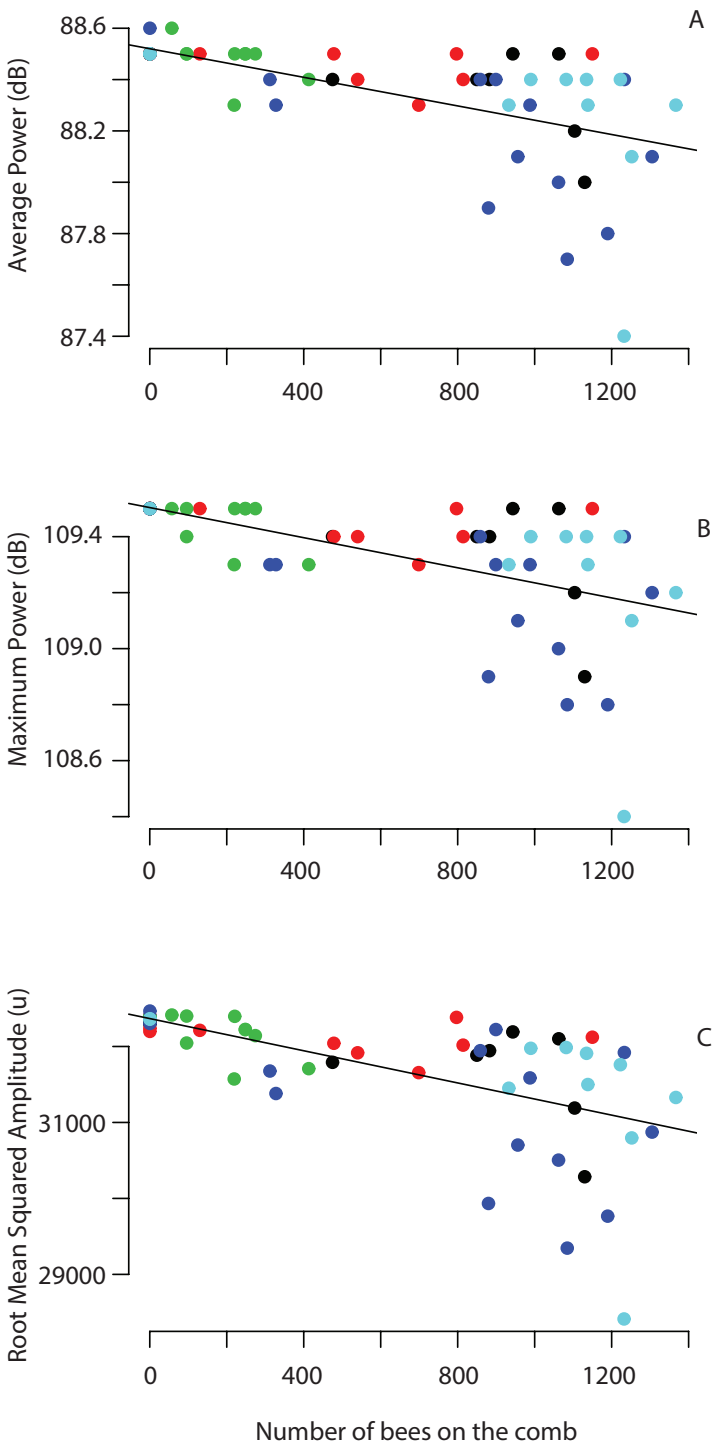

Figure 4.2 Some acoustic parameters of the comb vibrations decrease significantly as the number of bees on the focal comb increases. Average power, maximum power, and root mean squared amplitude all decrease as the number of bees on the comb increases (A, B, C, respectively). Colored points denote different colonies; black line shows the statistically significant regression.

Dead Bees on Comb

We tested whether the decrease in average power, maximum power, and root mean squared amplitude was due to the bees' mass, by inserting freshly killed bees head first into the cells of a focal comb that had no live bees on it. Even when the focal comb had 1600 dead bees, the PSD plots were no different from the baseline (Figure 4.3A), and there was no difference in any of the acoustic parameters (aggregate entropy, average entropy, average power, maximum power, energy, center frequency, maximum frequency, maximum amplitude, or root mean squared amplitude, $P > 0.05$). Dead bees did not decrease vibrations like what was observed with live bees (Figure 4.3B-D). This shows that it was not simply the mass of the bees that changed the comb vibrations.

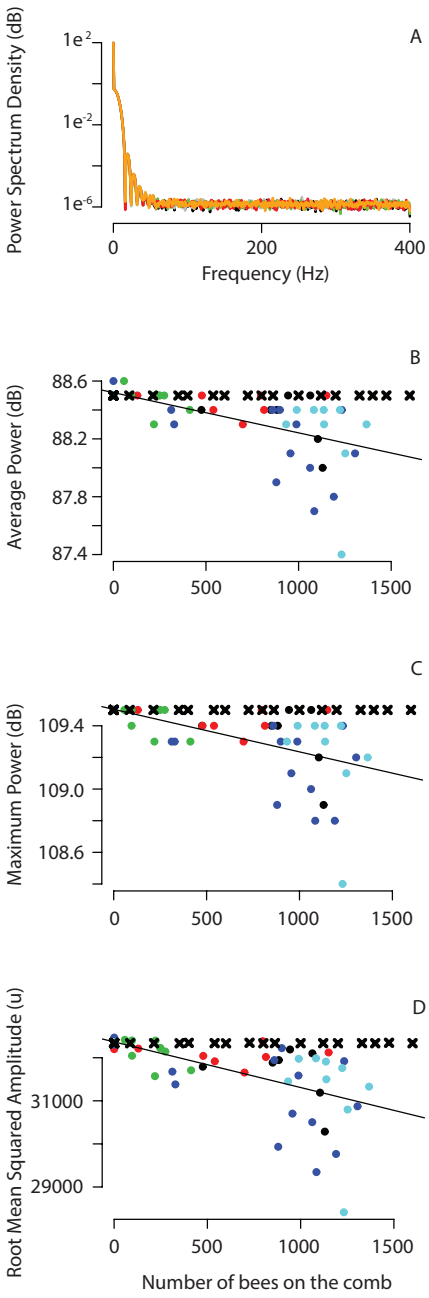

Figure 4.3 Comb vibrations did not change with the mass of bees on the comb. Power spectrum density plots showed no difference as the number of dead bees on the focal comb increases (A: black line, 0 dead bees on comb; red line, 400 dead bees; green line, 800 dead bees; orange line, 1200 dead bees; grey line, 1600 dead bees). Whereas comb vibrations decreased with the number of live bees, increasing the mass of the comb by adding dead bees did not reduce comb vibrations (B-D). X's in black denote the number of dead bees in the cells of the focal comb. Circles denote live bees, with colors indicating different colonies, and the black line showing the significant relationship between vibrations and live bees on the comb.

Time of Day

We tested whether comb vibrations changed with time of day. Using measurements taken throughout the day (at least five measurements per colony, spaced evenly between 06:00 and 24:00 h), we found that there was no significant difference in aggregate entropy, average entropy, average power, maximum power, energy, center frequency, maximum frequency, maximum amplitude, or root mean squared amplitude ($P > 0.05$). In summary, we found no influence of time of day on comb vibrations, indicating that the bees are not preferentially reducing comb vibrations during the day, or at night.

DISCUSSION

This study shows that as the number of bees on a comb increases, the amplitude of comb vibrations decrease. Average power, maximum power, and root mean squared amplitude all significantly decreased with an increasing number of workers on the comb (Figure 4.2). Surprisingly, the decrease in comb vibrations is not due to the mere mass of the bees, because adding dead bees to the comb had no effect on vibrations (Figure 4.3). Therefore, live bees actively damp comb vibrations, and the damping increases with worker number. By reducing the power of background vibrations, workers effectively make the comb "quieter," and improve the substrate for transmitting vibrations.

How do bees damp comb vibrations? While it's not their mass (Figure 4.3), it could be their posture. Whereas dead bees had to be inserted into the cells of the comb,

live bees tend to remain on the surface of the comb cells, even when sleeping (Klein et al. 2008). When a bee is on the comb, whether still or in motion, her tarsi are in contact with neighboring cells (red arrows in Figure 4.4). By connecting up to six different cells with her legs, she may act like a "bee staple" between cells, and reduce vibrations. Bees may even further damp vibrations by keeping their balance while on the comb, thus actively counteracting comb vibrations. While each bee only damps comb vibrations a bit, they collectively make a significant impact.

Figure 4.4 A bee standing on the comb, with her tarsi touching different comb cells (indicated by red arrows). The bee's posture may act like a staple, connecting neighboring cells, and damping comb vibrations.

Bees only begin investing in reproduction after surpassing a threshold colony size (Smith et al. 2014), so could workers use this reduction in comb vibrations to assess their colony's size? Probably not. While we know that workers monitor colony size, and only invest in reproduction once the colony surpasses a threshold colony size (Smith et al. 2014), the change in comb vibrations that occurs with increased colony size is likely too small for the bees to detect these differences. Average power, for example, is 88.52 dB with no bees on the comb, 88.49 dB with 100 bees, and 88.25 dB with 1000 bees (Figure 4.2A). Therefore, an individual would have to detect a 0.24 dB decrease in comb vibrations to distinguish being 1 of 100 versus 1 of 1000 bees on a comb. For known signals transmitted through comb, the difference between signal and noise is 5.8 ± 2.7 dB (Nieh and Tautz 2000), which demonstrates the scale needed for signals to overcome background noise. This is an order of magnitude greater than the damping effect that we found. Furthermore, Sandeman et al (Sandeman et al. 1996) performed extracellular recordings in honey bee legs placed on vibrating combs, and found that the minimum displacement that legs detected was 2 μm. When comb vibrations were highly damped by the bees, the difference in comb vibrations was only 0.05 μm (standard deviation of the vibrations: 0.22 μm with 0 bees on the comb; 0.17 μm with 1232 bees on the comb, recordings from 28 and 29 August 2016 at 12pm, respectively). This difference is two orders of magnitude smaller than vibrations that induce an electrophysiological response. Therefore, although bees reduce comb vibrations, we would not predict them to use this change in vibrations to detect their colony's size. This is not to say

that bees aren't detecting the background noise, but simply that they do not detect the *difference* in comb vibrations when 1, 100, or 1000 bees are on the comb.

For all organisms that communicate, a key challenge is ensuring that the signal will make its way through the noisy environment to reach its intended recipient. Male wolf spiders, *Schizocosa ocreata*, for example, seek out leaf litter to send courtship signals, which have higher signal efficacy than rock or soil (Gordon and Uetz 2011). In a honey bee colony, increasing the number of bees actually reduces background noise, which improves the landscape for transmitting substrate-borne vibrations between individuals. Indeed, the freezing response, where bees stop moving in response to substrate vibrations, was proposed to reduce background noise during queen communication (Michelsen et al. 1986a), but there is no evidence that the bees' freezing helps propagate the signal (Michelsen et al. 1986b). Our findings show that simply having more bees in the colony reduces background noise, regardless of whether the bees are moving or not. And, surprisingly, it is not just the mass of the bees that reduces vibrations, but their interactions with the comb that damps the vibrations. While we propose the "bee staple" mechanism for how honey bees actively damp comb vibrations, this hypothesis remains to be tested. What has been demonstrated, however, is that as a colony grows, so too does the communication landscape that is used for sending signals within the superorganism.

KEYWORDS

Substrate-borne vibration, signal propagation, honeycomb, superorganism, social insects, colony size

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REFERENCES

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67:1–48

Bencsik M, Le Conte Y, Reyes M, Pioz M, Whittaker D, Crauser D, Simon-Delso N, Newton MI (2015) Honeybee colony vibrational measurements to highlight the brood cycle. PLOS ONE 10:e0141926

Charif RA, Waack AM, Strickman LM (2010) Raven Pro 1.4 User's Manual. Cornell Lab of Ornithology, Ithaca, NY

Cocroft RB (1996) Insect vibrational defense signals. Nature 382:679–80

Cocroft RB, Rodríguez RL (2005) The behavioral ecology of insect vibrational communication. Bioscience 55:323–34

Gordon SD, Uetz GW (2011) Multimodal communication of wolf spiders on different substrates: Evidence for behavioural plasticity. Anim Behav 81:367–75

Hunt JH, Richard FJ (2013) Intracolony vibroacoustic communication in social insects. Insect Soc 60:403–17

Kirchner WH (1993) Acoustical communication in honeybees. Apidologie 24:297–307

Klein BA, Olzsowy KM, Klein A, Saunders KM, Seeley TD (2008) Caste-dependent sleep of worker honey bees. J Exp Biol 211:3028–40

Lewis F, Butler A, Gilbert L (2011) A unified approach to model selection using the likelihood ratio test. Methods Ecol Evol 2:155–62

Michelsen A, Kirchner WH, Andersen BB, Lindauer M (1986a) The tooting and quacking vibration signals of honeybee queens: a quantitative analysis. J Comp Physiol A 158:605–11

Michelsen A, Kirchner W, Lindauer M (1986b) Sound and vibrational signals in the dance language of the honeybee, *Apis mellifera*. Behav Ecol Sociobiol 18:207–12

Miklas N, Čokl A, Renou M, Virant-Doberlet M (2003) Variability of vibratory signals and mate choice selectivity in the southern green stink bug. Behav Processes 61:131–42

Nieh JC (2010) A negative feedback signal that is triggered by peril curbs honey bee recruitment. Curr Biol 20:310–5

Nieh JC, Tautz J (2000) Behaviour-locked signal analysis reveals weak 200-300 Hz comb vibrations during the honeybee waggle dance. J Exp Biol 203:1573–9

Pierce AL, Lewis LA, Schneider SS (2007) The use of the vibration signal and worker piping to influence queen behavior during swarming in honey bees, *Apis mellifera*. Ethology 113:267–75

R Core Team (2014) R: A language and environment for statistical computing. Vienna, Austria

Roever C (2015) bspec: Bayesian spectral inference. The Comprehensive R Archive Network. https://CRAN.R-project.org/package=bspec.

Sandeman D, Tautz J, Lindauer M (1996) Transmission of vibration across honeycombs and its detection by bee leg receptors. J Exp Biol 199:2585–94

Schneider SS, Painter-Kurt S, Degrandi-Hoffman G (2001) The role of the vibration signal during queen competition in colonies of the honeybee, *Apis mellifera*. Anim Behav 61:1173–80

Seeley TD, Reich AM, Tautz J (2005) Does plastic comb foundation hinder waggle dance communication? Apidologie 36:513–21

Smith ML, Koenig PA, Peters JM (2017) The cues of colony size: how honey bees sense that their colony is large enough to begin to invest in reproduction. J Exp Biol 220:1597–605

Smith ML, Ostwald MM, Loftus JC, Seeley TD (2014) A critical number of workers in a honeybee colony triggers investment in reproduction. Naturwissenschaften 101:783–90

Smith ML, Ostwald MM, Seeley TD (2016) Honey bee sociometry: tracking honey bee colonies and their nest contents from colony founding until death. Insect Soc 63:553–63

Suryanarayanan S, Hermanson JC, Jeanne RL (2011) A mechanical signal biases caste development in a social wasp. Curr Biol 21:231–5

Tautz J (1996) Honeybee waggle dance: recruitment success depends on the dance floor. J Exp Biol 1381:1375–81

Tautz J, Rohrseitz K (1998) What attracts honeybees to a waggle dancer? J Comp Physiol A 183:661–7

von Frisch K (1967) The Dance Language and Orientation of Bees. Belknap Press of Harvard University Press, Cambridge, MA